Shelf−life Estimation of Frankfurter Sausage Containing Dietary Fiber from Rice Bran Using Predictive Modeling

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예측미생물을 이용한 미강식이섬유 함유 프랑크푸르터 소시지의 유통기한 설정

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Abstract

Predictive modeling was applied to study the growth of microorganisms related to spoilage in frankfurter sausage containing various levels of dietary fiber (0, 1, 2, and 3%) from rice bran and to estimate its shelf-life. Using the Baranyi model, total viable cells, anaerobic and psychrotrophic bacteria were measured during 35 days of cold storage ($< 4\pm1$ºC). The lag times (LT) demonstrated by control and treatment groups were 6.28, 623, 6.24, and 6.25 days, respectively. The growth rate of total viable cells in each group were 0.95, 0.91, 0.92, and 0.91 (Log CFU/g/day), respectively. The anaerobic and psychrotrophic bacteria had lower initial (y_0) and maximal bacterial counts (y_{max}) than total viable cells. Also, the anaerobic and psychrotrophic bacteria possessed lower growth rate and longer lag time than total viable cells. The estimated shelf-life of frankfurter containing rice bran fiber by the growth rate of total viable cells was 7.8, 7.9, 7.9, and 7.7 days, respectively. There were no significant differences in shelf-life as a function of fiber content. In other words, the addition of dietary fiber in sausage did not show the critically hazardous results in growth of microorganism. The 12 predictive models were then characterized by high R^2, and small RMSE. Furthermore, B_f and A_f values showed a very close relationship between the predictive and observed data.

Key words : frankfurter sausage, predictive modeling, Baranyi function, dietary fiber, shelf-life

Introduction

Changes in eating habits and life styles have contributed significantly to the increased numbers of individuals who are overweight or obese in modern society (Aranceta, 2003). There has also been a significant increase in the number of fast food restaurants and amount of inexpensive food at supermarkets, possibly coinciding with certain life-style change, such as the increase in alcohol consumption, and sedentary habits (Martin *et al.*, 2008). However, the demands of high-quality and distribution of well-fitted nutrition on meat products are still increasing. Therefore, people and society are concerned with the obesity, high blood pressure, cancer, and heart disease seriously (Chin *et al.*, 2005).

In recent years, researchers have been interested in understanding the impact of dietary fiber in solving these emerging health problems (Abdul-Hamid and Luan, 2000). Intake of dietary fiber has positive effects against chronic diseases, such as cardiovascular diseases, diverticulosis, diabetes and colon cancer (Cara *et al.*, 1992; Wrick *et al.*, 1983). Dietary fiber also affects the functional properties of food (Elleuch *et al.*, 2008). Dietary fiber can be added to foods (meat, bakery products) to modify textural properties, avoid syneresis, and stabilize high fat food and emulsions (Abdul-Hamid and Luan, 2000; Choi *et al.*, 2007; Wang *et al.*, 2002).

The microbiological safety of food should be considered as well as its nutrition and functionality. However, the scientific vulnerability of shelf-life estimation by the companies themselves was indicated by the government. Therefore, KFDA (Korean Food and Drug Administration) has established new rule(s) for estimating the shelf-

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life of food products to present the scientific proof and improve the reliability of the safe food (KFDA, Notification No. 2007-66). Thus, the food industry should present scientific evidence such as physical, sensory, and microbiological properties of newly developed products to verify shelf-life.

Predictive modeling has been used to estimate the growth of bacteria related to spoilage of foods in order to calculate the shelf-life or specific pathogenic bacteria in food for quantitative risk assessments. Specific spoilage organisms are selected and used as test organisms: i.e. *Brochothrix thermosphacta* (Baranyi *et al.*, 1995). For example, predictions of *Listeria monocytogenes* growth in sesame leaves were based on environment factors (Park *et al.*, 2007) and the probable intake time of ready-to-eat *Kimbap* in market was calculated by using the Food MicroModel based on Gompertz model (Lee *et al.*, 2004).

The purpose of this study was to apply predictive modeling to study the growth of microorganisms related to spoilage of frankfurter sausage containing various levels of dietary fiber (rice bran at 0, 1, 2, or 3%) and estimate its shelf-life more reliably.

Materials and Methods

Extraction of dietary fiber from rice bran

Dietary fiber from rice bran was extracted by the modified AOAC enzymatic-gravimetric method (AOAC, 1995). Rice bran of a Japonica rice cultivar (*Oriza sativa* L.) was purchased from local market in Seoul. This rice bran was ground in a mill and passed through a 25 mesh sieve. The ground rice bran roasted at a temperature of about 105°C, and then defatted with hexane (*n*-hexane 95%) using a shaker (BS-11, Lab Companion, Korea) overnight. The defatted rice bran was gelatinized with 0.6% termamyl (LS, Novozymes, Denmark) at 95°C for 1 hr to remove starches, and then filtered. The residue was then washed three times using four volumes of heated water (100°C) and was allowed to equilibrate to room temperature (20°C, 6 hr). The residue was then washed with 99.9% ethanol (preheated to 60°C), and then filtered. The residue was then dried overnight in an air oven (55°C) and then cooled. The dietary fiber extracted from rice bran was placed in polyethylene bags and vacuum packaged using a vacuum packaging system (FJ-500XL, Fujee Tech, Seoul, Korea). The extracted fiber was then stored at 4°C until required for product manufacture.

Preparation of frankfurter sausage

Pork ham and pork back fat were purchased from a local market in Seoul. All subcutaneous and intramuscular fat and connective tissue were removed from meat. Lean pork and fat were ground through an 8 mm plate. Each batch of samples consisted of four meat batters which differed in their composition by adding rice bran extracts (0, 1, 2, and 3%). Four different types of frankfurter were formulated with sugar (CJ Co., Seoul, Korea), MSG (CJ Co., Seoul, Korea), garlic powder (Dongbang Food Master Co., Seoul, Korea), onion powder (Dongbang Food Master Co., Seoul, Korea) and sodium nitrite (Daesan Chemicals Co., Cheonan, Korea) (Table 1). Raw meat was homogenized and ground for 1 min in a silent cutter (Cutter Nr-963009, Scharfen, Germany). 1.5% NaCl (CJ Co., Seoul, Korea) and 0.2% sodium triployphosphate (Samchum Purechemical Co., Ltd., Seoul, Korea) previously dissolved in water and chilled (2°C) was then added to the meat mixture and mixed for 1 min. After mixing, the rice bran extract (0, 1, 2, or 3%) was added and batters were homogenized for 1 min. The emulsion was maintained temperatures < 10°C and a temperature probe (Kane-May, KM330, Germany) was used to monitor the temperature during this process. After emulsifying, meat batter was stuffed into collagen casing (#240, NIPPI Inc., Tokyo, Japan; approximate diameter of 25 mm) using a stuffer (Stuffer IS-8, Sirman, Italy). The frankfurters were then heated at 75°C for 30 min in a water bath (model 10-101, Dae Han Co., Korea). Ten kg samples of each frankfurter were prepared using this method. Microbiological analysis was performed over a period of 35 days of storage at 4±1°C.

Table 1. The formulation of frankfurter sausage containing dietary fiber

Ingredients		Formulation (%, batch)			
		C [1]	T1 [2]	T2 [3]	T3 [4]
Main material	Pork ham	50	50	50	50
	Pork fat	25	25	25	25
	Water	25	25	25	25
Additive	Dietary fiber	0	1.0	2.0	3.0
	Salt	1.5	1.5	1.5	1.5
	Sodium nitrite	0.01	0.01	0.01	0.01
	Phosphate	0.2	0.2	0.2	0.2
	MSG [5]	0.08	0.08	0.08	0.08
	Onion powder	0.05	0.05	0.05	0.05
	Garlic powder	0.05	0.05	0.05	0.05
	Sugar	0.5	0.5	0.5	0.5

[1]Control, [2]frankfurter with 1% fiber added, [3]frankfurter with 2% fiber added, [4]frankfurter with 3% fiber added, [5]Monosodium L-glutamate.

Microbiological analysis of frankfurter sausages

For bacterial counts in frankfurter sausage, bacteriological analytical manual was used (BAM, 2003). To determine the bacterial counts for each sample, 10 g of sausage was aseptically transferred into a sterile stomacher bag and 100 mL of sterile 0.1% peptone water (Difco, USA) was added. The sample was then homogenized in a stomacher (Masticator-Paddle-Blender, IUL Instrument, Spain) for 2 min at normal speed and aliquots were plated out directly as 10-fold dilutions in 0.1% peptone water. After serially diluting each sample in sterile peptone water, 0.1 mL portions of the samples were separately plated onto each of plates. Total viable cells and anaerobic bacterial counts were enumerated by incubation on plate count agar (PCA; Difco, Sparks, MD, USA) at 35ºC for 48 hr and anaerobic bacteria was determined by spread-plating on PCA using AnaeroGen (Oxoid, Hampshire, UK) and BBL anaerobic jar (Difco, USA) at 35ºC for 48 hr. Psychrotrophic bacteria were incubated at 10ºC for 7 days on PCA. After incubation such plates, which contained 30 to 300 colonies on a plate, were chosen for counting. All analyses were performed three times and counts were expressed as colony-forming units per gram (CFU/g).

Predictive models of the bacterial growth in frankfurter sausage

The growth data were analyzed with widely used Baranyi model (Baranyi *et al.*, 1995; Baranyi *et al.*, 1996). Baranyi model has been reparameterized in terms of more familiar quantities. The reparameterized model is described by form (1), (2), and (3).

$$y(t) = y_0 + \frac{y_1}{\ln(10)} - \frac{y_2}{\ln(10)} \tag{1}$$

$$y_1 = \mu \cdot t + \ln[e^{-\mu \cdot t} - e^{-\mu(t+t_{lag})} + e^{-e \cdot t_{lag}}] \tag{2}$$

$$y_2 = \ln[1 + 10^{(y_0 - y_{max})} \cdot (e^{\mu(t-t_{lag})} - e^{-\mu \cdot t_{lag}})] \tag{3}$$

Where $y(t)$ is the bacterial count in log CFU/g at time t; y_0 is the initial bacteria count in Log CFU/g at time 0; y_{max} is the maximum bacteria count in Log CFU/g; t_{lag} means lag time (LT); μ_{max} is the maximum specific growth rate, Log CFU/g/day. The average parameters of y_0, y_{max}, LT, μ_{max} in this study were determined by using the MicroFit version 1.0 (developed by the Institute of Food Research, Norwich, UK).

Evaluation of predictive models

The indices used for comparisons of predicted and observed data were: the determination coefficient R^2, modified bias factors, accuracy factors (B_f, A_f, respectively), and root mean square error (RMSE) (Baranyi *et al.*, 1996; Ross *et al.*, 2000).

$$B_f = 10^{\left(\frac{\Sigma \log(pred/obs)}{n}\right)} \tag{4}$$

$$A_f = 10^{\left(\frac{\Sigma |\log(pred/obs)|}{n}\right)} \tag{5}$$

$$RMSE = \sqrt{\frac{\Sigma(obs-pred)^2}{n}} \tag{6}$$

Where *obs* means observed value; *pred* means predicted value; n is the number of observations. Perfect agreement between predictions and observations leads to bias and accuracy equal to 1.0. If A_f value higher than 1, the A_f indicates that predicted values are larger than observed values. RMSE is effectively 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)).

Estimation of shelf-life

The shelf-life of frankfurter sausage was calculated by the enumeration of total viable cells. 4 Log CFU/g is the critical level of total viable cells allowed in cooked meat products as specified by the Korean Food Standards Codex (Korean Food and Drug Administration, 2008). Thus the time to reach the limit of 4 Log CFU/g, which was calculated from primary models, was denoted as the predicted shelf-life. And the estimated shelf-life (Equation (7)) is calculated by taking the product of the predicted shelf-life times the safety factor (Koo *et al.*, 2007).

Estimated shelf-life
= Predicted shelf-life × Safety factor (1/1.5)　　(7)

Korean Health Industry Development Institute (KHIDI) denoted that the safety factor was the probable time, being able to maintain the quality of target products and the commercial shelf-life (mentioned as the estimated shelf-life) of products should reflect safety factor (KHIDI, 2008). The safety factor can be changeable depending on purpose and type of foods. There is not enough data about safety factor now. Therefore we used the value of safety factor (1/1.5) which was recommended on ready-to-eat food (Koo *et al.*, 2007).

Statistical analysis

An analysis of variance (ANOVA) was performed on

all the variables measured using the general linear model (GLM) procedure of the SAS program (Statistics Analytical System, USA, 1999). The Duncan's multiple range tests was used to determine differences between treatment means.

Results and Discussion

Application of predictive models on bacterial growth in frankfurter sausage

On the basis of the observed data, predictive models were described by the Baranyi model. Fig. 1-3 shows total viable cells, anaerobic, and psychro- trophic bacterial predictive models in frankfurter sausage containing different dietary fiber levels, respectively. Table 2 shows the estimated growth parameters, y_0, y_{max}, μ_{max}, and LT. The numbers of heat-stable bacteria were below detectable levels and coliforms were not detected in frankfurter samples (data not shown). Fig. 1 (A)-(D) show predictive models and experimental data points (n=8) of total viable cells during 35 days of storage. Control and treatments for total viable cells had lag times of 6.28, 623, 6.24, and 6.25 days, respectively. After this lag time, bacterial counts increased between 7 to 21 days of storage, reaching a maximal population (y_{max}) from 7.88 to 8.59 Log CFU/g. After 35 days of storage, the counts of total viable cells maintained the constant levels of bacterial counts. Lag time values were not significantly different ($p>0.05$). The growth rate of total viable cells showed 0.95, 0.91, 0.92, and 0.91 Log CFU/g/day, respectively. There were no significant differences in growth rates between control and treatment groups (T1, T2, and T3; $p>0.05$).

Anaerobic bacteria had lower initial (y_0) and maximal bacterial counts (y_{max}) compared to counts of total viable cells. Also growth rate values, 0.65, 0.68, 0.67, and 0.70 Log CFU/g/day were lower than the growth rate values of total viable cells (Table 2). There were no significant differences in growth rates among control, T1, T2, and T3 groups ($p>0.05$). However, lag times in anaerobic bacteria samples were longer than in total viable cells (i.e. 9.63, 9.10, 7.70, and 9.18 (days), respectively).

Fig. 3 (A)-(D) describe the predictive models of psychrotrophic bacteria in frankfurter samples. Psychrotrophic bacteria growth curves showed that control, T1, T2, and T3 were similar to total viable cells and anaerobic bacterial counts. The values of initial and maximum counts of control, T1, T2, and T3 groups showed no sig-
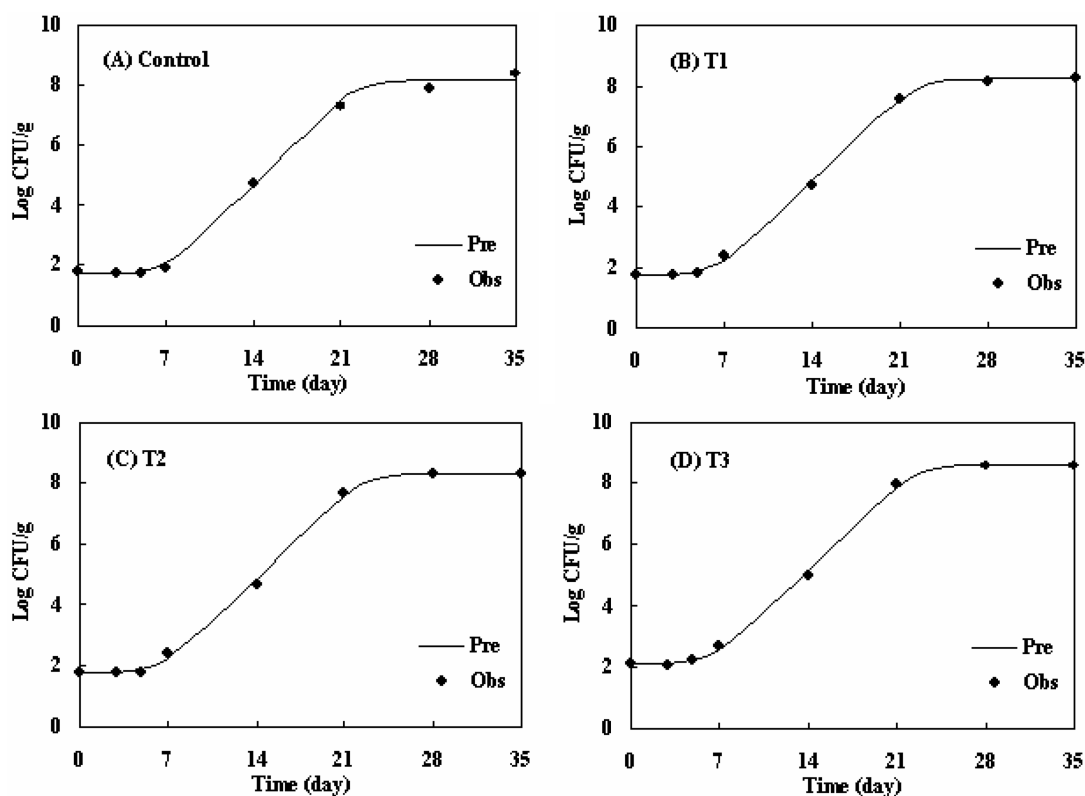

Fig. 1. Predictive models of total viable cells generated by the Baranyi model in frankfurter sausage containing dietary fiber during 35 days of storage at 4±1°C (—, predicted data; ●, observed data).
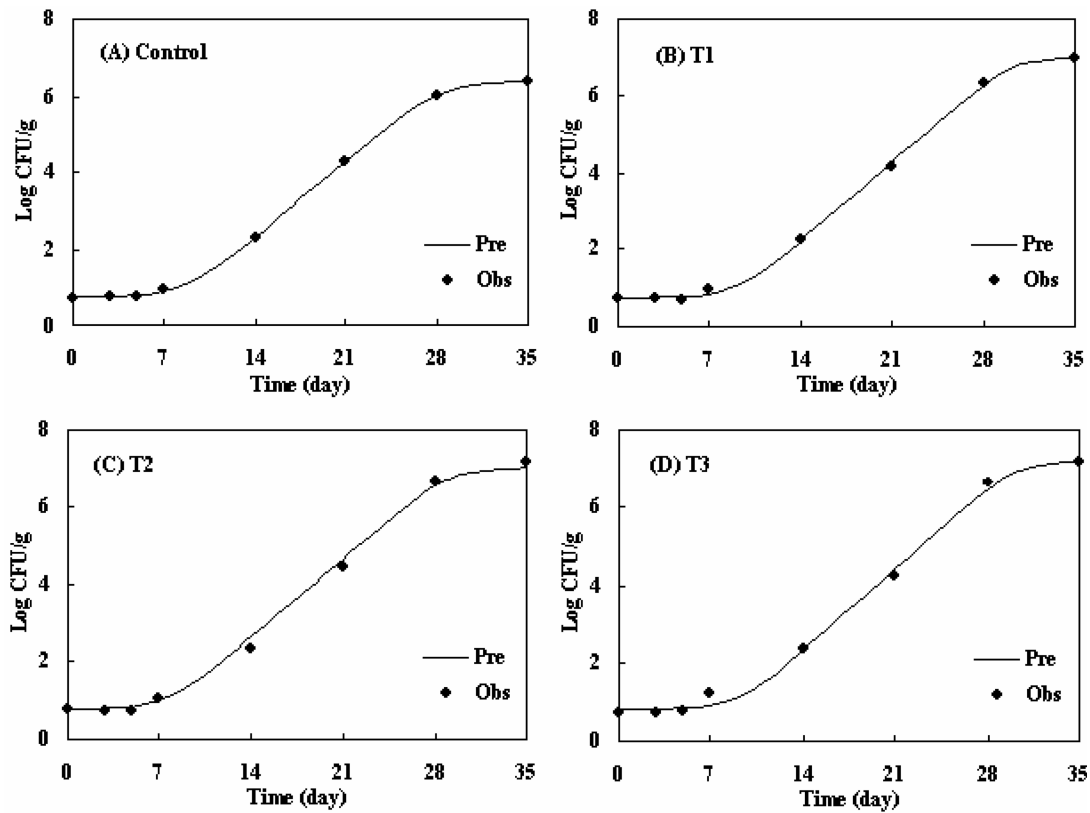
Fig. 2. Predictive models of anaerobic bacteria generated by the Baranyi model in frankfurter sausage containing dietary fiber during 35 days of storage at 4±1°C (—, predicted data; ●, observed data).

Fig. 3. Predictive models of psychrotrophic bacteria generated by the Baranyi model in frankfurter sausage containing dietary fiber during 35 days of storage at 4±1°C (—, predicted data; ●, observed data).
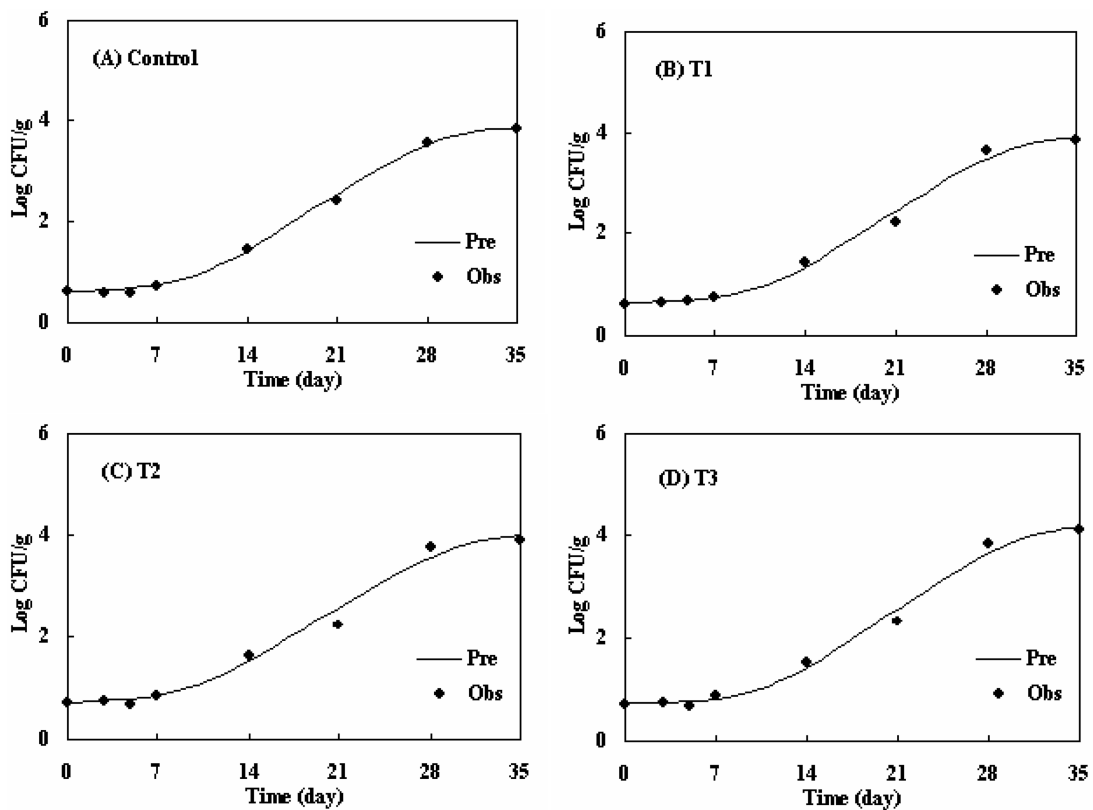
Table 2. Growth parameters of microorganisms as predicted by the Baranyi model in frankfurter sausage containing dietary fiber

Microorganism	Type	Growth parameter (mean±S.D.)			
		y_0 [5]	y_{max} [6]	μ_{max} [7]	LT [8]
Total viable cells	C [1]	1.76 ± 0.04^b	8.38 ± 0.05^b	0.95 ± 0.02^a	6.28 ± 0.28^a
	T1 [2]	1.78 ± 0.08^b	8.25 ± 0.08^b	0.91 ± 0.04^a	6.23 ± 0.50^a
	T2 [3]	1.76 ± 0.08^b	8.28 ± 0.08^b	0.92 ± 0.04^a	6.24 ± 0.52^a
	T3 [4]	2.08 ± 0.07^a	8.59 ± 0.08^a	0.91 ± 0.04^a	6.25 ± 0.48^a
Anaerobic bacteria	C	0.77 ± 0.02^a	6.39 ± 0.04^b	0.65 ± 0.01^a	8.63 ± 0.27^{ab}
	T1	0.74 ± 0.06^a	7.02 ± 0.01^a	0.68 ± 0.03^a	9.10 ± 0.54^a
	T2	0.76 ± 0.07^a	7.04 ± 0.12^a	0.67 ± 0.03^a	7.70 ± 0.71^b
	T3	0.82 ± 0.12^a	7.2 ± 0.23^a	0.70 ± 0.06^a	9.18 ± 1.16^a
Psychrotrophic bacteria	C	0.61 ± 0.03^a	3.90 ± 0.07^a	0.40 ± 0.02^a	10.01 ± 0.64^a
	T1	0.64 ± 0.09^a	3.95 ± 0.18^a	0.40 ± 0.05^a	10.73 ± 1.62^a
	T2	0.71 ± 0.13^a	4.04 ± 0.28^a	0.38 ± 0.07^a	9.90 ± 2.48^a
	T3	0.72 ± 0.10^a	4.22 ± 0.22^a	0.41 ± 0.06^a	10.81 ± 1.74^a

[1]Control, [2]frankfurter with 1% fiber added, [3]frankfurter with 2% fiber added, [4]frankfurter with 3% fiber added, [5]predictive initial cell count (Log CFU/g), [6]predictive maximum cell count (Log CFU/g), [7]maximum specific growth rate (Log CFU/g/day), [8]lag time (days).

nificant differences ($p>0.05$), and growth rates were lower than for total viable cells and anaerobic bacteria. The growth rate values were 0.40, 0.40, 0.38 and 0.41 (Log CFU/g/day). The values were not significantly different ($p>0.05$). Until now, usually the research has been studied about the effect of dietary fiber on food texture. Thus there are not enough literatures to compare and estimate the effect of dietary fiber on growth of microorganism in meat products. These results indicate that the contents of dietary fiber did not influence the growth rate and lag time. In other words, the addition in the range of 1, 2, and 3% of dietary fiber in sausage did not show the critically hazardous results, y_{max}, μ_{max}, and LT, comparing with control.

Evaluation of predictive models

Traditionally, the source of foods to influence the growth of spoilage or pathogenic bacteria has been determined by inoculating with interested bacteria. However, the gap between the predicted data and the observed data still exists. Because the predicted data made under the aqueous phase such as broth, it showed usually faster growth than the observed data made under structured phase like sausages or meat (Koutosoumanis and Nychas, 2000; Wilson et al., 2002). To overcome this problem, we used other method that is using data obtained from experiments conducted on natural products. Therefore, these models may show more accurately the growth of bacteria in frankfurter containing dietary fiber. To evaluate the predictive models, the indices used for comparisons of predicted and observed data were B_f, A_f, RMSE, and R^2.

Twelve predictive models were characterized by a high determination coefficient and small RMSE (Table 3). The data obtained for samples stored at 4 ± 1^oC was fitted into Baranyi model and graphically very well. However, R^2 values are not recommended with non-linear regression to judge model performance as absolute indices (Ross, 1996). Thus B_f and A_f were recommended (Baranyi et al., 1996). For total viable cells, B_f and A_f values of control were 1.004 and 1.016, respectively (perfect agreement would be 1.0). The B_f of 1.004 indicates that the predictive bacterial counts exceeded the observed counts by approximately 0.4%. Therefore, the values of B_f, A_f, RMSE, and R^2 statistics indicated that the Baranyi model could provide accurate predictions of growth data. The statistical indices of anaerobic and psychrotrophic bacteria also showed that the Baranyi model was reliable enough to describe bacterial growth curves in our frankfurter samples. Although we made models under real foods, there are not enough data to compare the effect of dietary fiber on bacterial growth in meat products. Thus, the experiments continuously should be tried to obtain data on the effect of dietary fiber or other factors such as water activity, pH, and water contents on the bacterial growth.

Estimation of shelf-life

The critical level of bacteria related to spoilage or food-born disease in cooked meat products varies extensively, therefore we followed the Korean Food Standards Codex (Korean Food and Drug Administration, 2008). The Korean Food Standards Codex regulates the critical level

Table 3. Evaluation of the predictive model against experimental data of frankfurter sausage containing dietary fiber

Microorganism	Type	Statistical analysis			
		$R^{2\ 5)}$	RMSE [6]	B_f [7]	A_f [8]
Total viable cells	C[1]	0.999	0.05	1.004	1.016
	T1[2]	0.997	0.08	1.002	1.021
	T2[3]	0.996	0.08	1.001	1.022
	T3[4]	0.997	0.08	0.999	1.015
Anaerobic bacteria	C	0.994	0.03	1.005	1.022
	T1	0.999	0.07	1.000	1.042
	T2	0.961	0.09	1.027	1.065
	T3	0.989	0.16	1.007	1.099
Psychrotrophic bacteria	C	0.985	0.04	1.039	1.045
	T1	0.986	0.11	1.014	1.045
	T2	0.989	0.15	1.029	1.062
	T3	0.984	0.12	1.029	1.062

[1]Control, [2]frankfurter with 1% fiber added, [3]frankfurter with 2% fiber added, [4]frankfurter with 3% fiber added, [5]correlation coefficient, [6]root mean square error, [7]bias factors; [8]accuracy factors.

Table 4. Evaluation of shelf-life of frankfurter sausage containing dietary fiber under 4±1°C storage

Microorganism	Type	Shelf-life (days)	
		Predicted shelf-life[5]	Estimated shelf-life[6]
Total viable cells	C[1]	11.7	7.8
	T1[2]	11.8	7.9
	T2[3]	11.9	7.9
	T3[4]	11.6	7.7

[1]Control; [2]frankfurter with 1% fiber added; [3]frankfurter with 2% fiber added; [4]frankfurter with 3% fiber added; [5]days to reach 10^4 CFU/g; [6]shelf-life estimated from equation (7).

of total viable cells (4 Log CFU/g) in cooked meat products. The shelf-life of frankfurters was assessed by measuring the time taken to reach the limit of 4 Log CFU/g of total viable cells under 4±1°C storage for 35 days. The values obtained from control, T1, T2, and T3 were compared each other (Table 4). The predicted shelf-life was 11.7, 11.8, 11.9, and 11.6 days. To calculate shelf-life more reliably, the predicted shelf-life was calculated again by considering the safety factor (1/1.5). The estimated shelf-life was 7.8, 7.9, 7.9, and 7.7 days. The shelf-life of frankfurter sausages was similar between control and treatments and appeared to be independent on the contents of dietary fiber.

According to the new rule(s) established by KFDA (Korean Food and Drug Administration, Notification No. 2007-66), the food company should present the shelf-life of food products and the proof such as scientific method of shelf-life estimation, and procedures. However the reliable standard experimental methods for the estimation of shelf-life depending on various food characteristics have not been developed sufficiently. The microbiological quality in food is concerned as food spoilage and safety undoubtedly these days. And also ensuring the microbiological safety on food is critical problem for food control and distribution. The mathematical predictive models of microorganism have been developed for understanding the bacterial growth related to spoilage or food borne disease (Dalgaard, 1995; Fujikawa *et al*., 2004). Therefore, the application of predictive modeling may be the reliable approach for estimation of shelf-life.

Acknowledgements

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