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Estimation of Shelf-life of Frankfurter Using Predictive Models of Spoilage Bacterial Growth

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Abstract

The aim of this research was to develop predictive models for the growth of spoilage bacteria (total viable cells, *Pseudomonas* spp., and lactic acid bacteria) on frankfurters and to estimate the shelf-life of frankfurters under aerobic conditions at various storage temperatures (5, 15, and 25°C). The primary models were determined using the Baranyi model equation. The secondary models for maximum specific growth rate and lag time as functions of temperature were developed by the polynomial model equation. During 21 d of storage under various temperature conditions, lactic acid bacteria showed the longest lag time and the slowest growth rate among spoilage bacteria. The growth patterns of total viable cells and *Pseudomonas* spp. were similar each other. These data suggest that *Pseudomonas* spp. might be the dominant spoilage bacteria on frankfurters. As storage temperature increased, the growth rate of spoilage bacteria also increased and the lag time decreased. Furthermore, the shelf-life of frankfurters decreased from 7.0 to 4.3 and 1.9 (d) under increased temperature conditions. These results indicate that the most significant factor for spoilage bacteria growth is storage temperature. The values of B_f, A_f, *RMSE*, and R^2 indicate that these models were reliable for identifying the point of microbiological hazard for spoilage bacteria in frankfurters.

Key words : frankfurter, predictive models, Baranyi model, polynomial model, shelf-life

Introduction

Frankfurters are one of the most widely consumed meat products world wide, and the extension of their shelf-lives is important for consumer safety and product distribution. Consumer' needs and market demands are focused increasingly on awareness of meat quality assurance and extended shelf-life. The Korean Food and Drug Administration (KFDA) had stated that it is necessary to make a new rule for setting the shelf-life of products (KFDA, Notification No. 2007-66). Thus, food manufactures should present scientific evidence pertaining to physical, sensory, and microbiological properties for newly developed products to determine their shelf-lives. Scientists and manufacturers are therefore developing quality control and safety assurance systems.

Predictive modeling is an important tool that has been introduced to predict the behavior of microorganisms under various environmental conditions such as temperature, pH, and a_w (Zurera-Cosano *et al.*, 2006). Furthermore, predictive modeling has been used to estimate the growth of spoilage microorganisms in order to determine the shelf life of food products. For example, specific microorganisms associated with food spoilage, such as *Brochothrix thermosphacta*, lactic acid bacteria (LAB), and *Pseudomonas* spp. were selected and modeled using the Baranyi model (Pin and Baranyi, 1998). In addition, the growth of total viable cells, H_2S-producing bacteria, and *Enterobacteriaceae* were determined by logistic model (Koutsoumanis and Nychas, 2000).

It was reported that spoilage of meat or meat products under aerobic conditions is due primarily to *Pseudomonas, Acinetobacter*, and *Psychrobacter* spp. (Pin and Baranyi, 1998). Under anaerobic conditions, spoilage is largely due to *Brochothrix thermosphacta* and LAB when their concentrations reach greater than 7 Log CFU/cm^2, or /g. However, *Brochothrix thermosphacta* and LAB can also grow in aerobically stored meat but show slower growth and delayed spoilage (Dainty and Mackey 1992; Gill and Penney, 1977; Lambropoulou *et al.*, 1996).

Manufacturers currently make handmade cooked frankfurters as a Ready-To-Eat food. The advantage of products under refrigeration and vacuum-packaging is that

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refrigeration under vacuum can inhibit the growth of phychrophiles and mesophiles (Kenny *et al.*, 2005). However, many handmade frankfurters are continuously displayed under aerobic conditions and temperature abuse conditions in local markets. Therefore, the aim of this study was to apply predictive modeling for the growth of spoilage bacteria (total viable cells, *Pseudomonas* spp., and LAB) on frankfurters stored aerobically at various temperatures and to reliably estimate frankfurter shelf-life to improve microbiological quality assurance.

Materials and Methods

Preparation of frankfurters

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 the meat. Lean pork and fat were ground through an 8 mm plate. Frankfurters 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 triploy phosphate (Samchum Purechemical Co., Seoul, Korea) previously dissolved in water and chilled (2°C) were then added to the meat mixture and mixed for 1 min and the meat batter was stuffed into collagen casings (#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). After heating and cooking, the frankfurters, which were cooled under the ambient temperature (<15°C), then stored at 5, 15, and 25°C and experimented for the storage period.

Table 1. Frankfurter formulation

Ingredients		Formulation (%)
Main material	Pork ham	50
	Pork fat	25
	Water	25
Additive	Salt	1.5
	Sodium nitrite	0.01
	Phosphate	0.2
	MSG [1)]	0.08
	Onion powder	0.05
	Garlic powder	0.05
	Sugar	0.5

[1)] Monosodium L-glutamate.

Microbiological analysis of frankfurters

The bacteriological analytical manual was used to determine bacterial counts in frankfurters (BAM, 2003). For each sampling, 10 g of frankfurter was aseptically transferred into a sterile stomacher bag and 100 mL of sterile 0.1% peptone water was added. The sample was then homogenized in the stomacher (Masticator-Paddle-Blender, IUL Instrument, 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 portions of the samples were plated separately on Plate Count Agar (PCA; Difco, USA) followed by incubation at 35°C for 48 h. The number of *Pseudomonas* spp. was estimated by incubation on Cetrimide Fusidin Cephaloridine agar with CFC supplements (CFC agar with supplements, Oxoid, UK) at 35°C for 48 h. LAB counts were determined on MRS agar (MRSA, Difco, USA) at 35°C for 48 h. After incubation, such plates which contained 30 to 300 colonies were chosen for counting. All analyses were performed three times and counts were expressed as colony-forming units per gram of frankfurter (CFU/g).

Experimental procedures

It was reported that predictive models developed in broth show faster bacterial growth than in natural products (Koutsoumanis and Nychas, 2000). Therefore, to develop reliable predictive models we directly conducted experiments on prepared frankfurters and applied the data to the Baranyi model. Prepared frankfurter was weighed (200 g) and aseptically transferred into three polyethylene bags for each temperature. To make the aerobic storage condition, frankfurters within polyethylene bags were not vacuum-packed. All samples were stored aerobically at a constant temperature of 5, 15, or 25°C for 21 d. Samples were tested at 0, 1, 2, 3, 5, 7, 9, 14, and 21 d of storage for each temperature.

Primary models of spoilage bacteria in frankfurters

The data were applied to the widely used Baranyi model (Baranyi and Roberts, 1994). The Baranyi model has been reparameterized in terms of more familiar quantities. The reparameterized model is described by forms (1), (2), and (3).

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

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

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

In these forms, $y(t)$ 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/h. The average parameters of y_0, y_{max}, LT, μ_{max} in this study were determined by using the program MicroFit version 1.0 (developed by the Institute of Food Research, Norwich, UK).

Secondary models of spoilage bacteria in frankfurters

To describe the effects of temperature (5, 15, and 25°C) on bacterial growth, the polynomial model equation was chosen based on the parameters of primary models. To describe the effects temperature on maximum specific growth rate (μ_{max}) and LT (lag time), the polynomial model equation was described in the following forms (4, 5).

$$\mathrm{Ln}(\mu_{max}) = a + b\mathrm{T} + c\mathrm{T}^2 \quad (4)$$

$$\mathrm{Ln}(\mathrm{LT}) = a + b\mathrm{T} + c\mathrm{T}^2 \quad (5)$$

a, b, and c are constants, and T is temperature (°C). To obtain the three constants (a, b, and c), all data were fitted using the nonlinear regression procedure, PROC NLIN, with SAS version 9.1.

Evaluation of predictive models

Goodness-of-fit of the predictive models (primary and secondary models) was evaluated using the coefficient of determination (R^2), modified bias factors, accuracy factors (B_f, A_f respectively), and root mean square error (*RMSE*).

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

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

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

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 values equal to 1.0. If the A_f value is 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, and has the same units as the data (typically Log CFU/g) (Equation (8)).

Estimation of shelf-life

According to the Korean Food Standards Codex, the number of total viable cells on cooked meat products should be limited to less than 4 Log CFU/g (KFDA, 2008). Therefore the shelf-life of frankfurters was calculated based on the enumeration of total viable cells. 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. The estimated shelf-life (Equation (9)) was 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) (9)

The Korean Health Industry Development Institute (KHIDI) denoted that the safety factor was the time required to maintain the quality of target products. The commercial shelf-life (mentioned as estimated shelf-life) should reflect the safety factor (KHIDI, 2008). The safety factor can change depending on the purpose and type of food. There is not enough data about safety factors at present, therefore we used the safety factor value (1/1.5) recommended for Ready-To-Eat foods (Koo *et al.*, 2007).

Statistical analysis

An analysis of variance was performed on all the variables measured using the general linear model (GLM) procedure of the SAS (SAS version 9.1). The Duncan's multiple range tests was used to determine differences between treatment means.

Results and Discussion

Predictive models of spoilage bacteria in frankfurters

Predictive models were developed by the Baranyi model based on experimental data. Figs. 1-3 show primary models of spoilage bacteria (total viable cells, *Pseudomonas* spp., and LAB) on frankfurters under various temperature conditions. Table 4 shows secondary models of the specific growth rate (μ_{max}) and lag time (LT) of spoilage bacteria. Table 2 shows the estimated

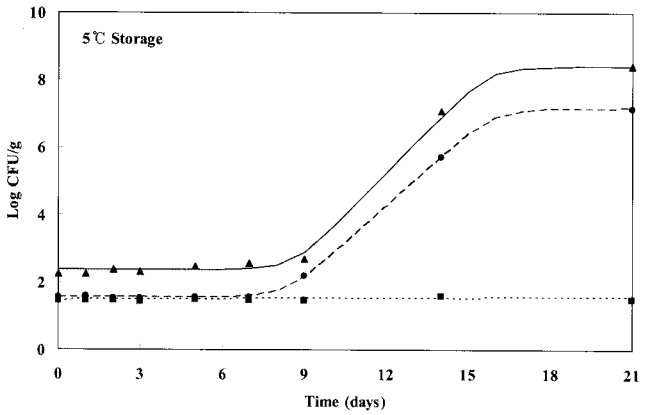

Fig. 1. Primary models of spoilage bacteria in frankfurters during 21 d of storage at 5°C (▲, total viable cells; ◆, *Pseudomonas* spp.; ■, lactic acid bacteria).

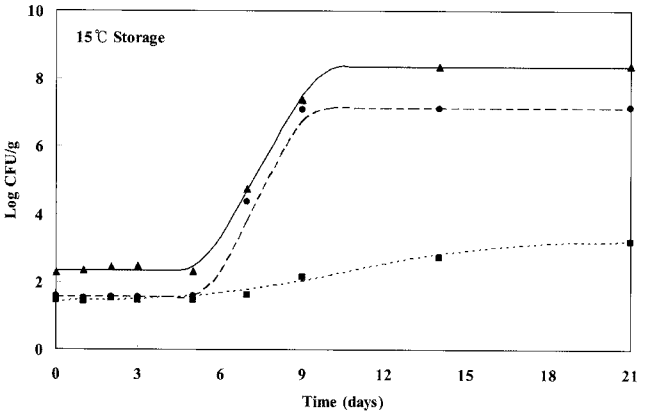

Fig. 2. Primary models of spoilage bacteria in frankfurters during 21 d of storage at 15°C (▲, total viable cells; ◆, *Pseudomonas* spp.; ■, lactic acid bacteria).

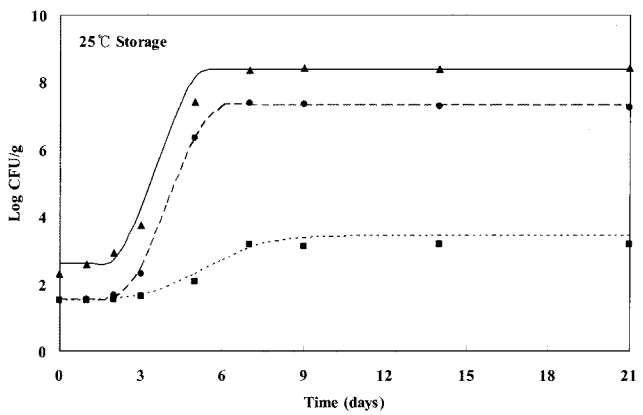

Fig. 3. Primary models of spoilage bacteria in frankfurters during 21 d of storage at 25°C (▲, total viable cells; ◆, *Pseudomonas* spp.; ■, lactic acid bacteria).

growth parameters; y_0, y_{max}, μ_{max}, and LT. The initial cell counts of each spoilage bacteria were 2.35-2.61 and 1.54-1.55 (Log CFU/g). The initial counts of *Pseudomonas* spp. were significantly less than the number of total viable cells ($p<0.05$). At 5°C, growth of LAB was not observed (Table 2), thus it was impossible to determine the predictive values. At the initial stage of bacterial growth (lag phase), the numerical differences between *Pseudomonas* spp. and LAB at 15 and 25°C storage temperatures were minimal. However, after the exponential phase the number of *Pseudomonas* spp. reached 7.12-7.33 (Log CFU/g). The maximum cell counts of LAB were approximately 3.21-3.43 (Log CFU/g) during the stationary phase. It was reported that a mixture of three *Pseudomonas*, two *Acinetobacter*, two *Psychrobacter*, and one *Shewanella* strains were the dominant microorganisms related to spoilage among 32 strains of spoilage bacteria in broth, and that these strains inhibited other spoilage bacteria (Pin and Baranyi, 1998). It has also been reported that *Pseudomonas* spp. among natural microorganisms play important roles in most cases of spoilage of meat stored aerobically at different temperatures (Koutsoumanis *et al.*, 2006). Table 2 shows that the growth rates of *Pseudomonas* spp. are significantly faster than the growth rates of LAB at 15 and 25°C storage tem-

Table 2. Growth parameters of spoilage bacteria on frankfurters

Temperature (°C)	Microorganisms	Growth parameter (Mean ± SD)			
		y_0 [1]	y_{max} [2]	μ_{max} [3]	LT [4]
5	T [5]	2.38 ± 0.05^a	8.40 ± 0.13^a	1.92 ± 0.25^a	8.56 ± 0.59^b
	P [6]	1.54 ± 0.01^b	7.16 ± 0.02^b	1.69 ± 0.02^b	8.30 ± 0.05^b
	L [7]	- [8]	-	-	-
15	T	2.35 ± 0.05^a	8.37 ± 0.07^a	3.32 ± 0.17^b	5.36 ± 0.15^b
	P	1.55 ± 0.01^b	7.12 ± 0.01^b	3.70 ± 0.45^a	5.61 ± 0.14^b
	L	1.42 ± 0.06^c	3.21 ± 0.12^c	0.51 ± 0.09^c	6.91 ± 0.97^a
25	T	2.61 ± 0.11^a	8.40 ± 0.09^a	4.49 ± 0.95^a	2.21 ± 0.35^b
	P	1.55 ± 0.04^b	7.33 ± 0.03^b	4.58 ± 0.11^a	2.56 ± 0.07^b
	L	1.51 ± 0.01^b	3.43 ± 0.01^c	1.22 ± 0.08^b	3.62 ± 0.11^a

[1] Initial cell count (Log CFU/g), [2] maximum cell count (Log CFU/g), [3] maximum specific growth rate (Log CFU/g/d), [4] lag time (d), [5] total viable cells, [6] *Pseudomonas* spp., [7] lactic acid bacteria, [8] data not observed.

peratures ($p<0.05$). During 21 d of storage at various temperatures, LAB had longer lag times and slower growth rates than the total viable cells and *Pseudomonas* spp. ($p<0.05$). All figures (Figs. 1-3) show that the growth tendencies of total viable cells and *Pseudomonas* spp. are similar to each other. These data suggest that *Pseudomonas* spp. might be the dominant spoilage bacteria in frankfurters.

Regarding specific growth rates and lag times, the storage temperature (5, 15, and 25°C) had an important influence on the growth of spoilage bacteria in frankfurters. In other words, both specific growth rate and lag time were significantly temperature-dependent ($p<0.05$). To describe the effects of temperature on bacterial growth parameters (the specific growth rate and lag time) the polynomial model was chosen (Equation (4, 5), Table 4). LAB did not show growth at 5°C, thus the secondary models were not determined. As the storage temperature increased, the specific growth rates of spoilage bacteria increased as well and the lag time shortened. Other scientific studies agree that the most significant factor for the growth of spoilage bacteria is storage temperature (Gospavic *et al.*, 2008; Neumeyer, Ross, and McMeekin, 1997; Pin and Baranyi, 1998).

Table 3. Evaluation of primary models against experimental data

Temperature (°C)	Microorganisms	Statistical analysis			
		R^2 [1]	*RMSE* [2]	B_f [3]	A_f [4]
5	T [5]	0.96	0.07	1.014	1.091
	P [6]	0.95	0.06	0.995	1.019
	L [7]	- [8]	-	-	-
15	T	0.94	0.05	1.007	1.054
	P	0.95	0.12	0.955	1.072
	L	0.93	0.09	0.957	1.072
25	T	0.92	0.13	1.077	1.118
	P	0.99	0.21	1.013	1.047
	L	0.93	0.17	1.109	1.141

[1] Correlation coefficient, [2] root mean square error, [3] bias factors, [4] accuracy factors, [5] total viable cells, [6] *Pseudomonas* spp., [7] lactic acid bacteria, [8] data not observed.

Evaluation of predictive models

To evaluate the predictive models, the indices used for comparisons of predicted and observed data were B_f, A_f, *RMSE*, and R^2. Table 3 and 4 indicate how well the primary and secondary models described the growth data used in model development. In every case, the predictive models produced high values for the determination coefficient (R^2) and small values for the *RMSE*. 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). Primary and secondary models show that these models are close to the ideal values (perfect agreement would be 1.0). Therefore, there was a good fit between predicted and observed data. The values of B_f, A_f, *RMSE*, and R^2 indicate that the Baranyi and polynomial models provide good fitness for the growth data, and these models were also reliable to describe the point of microbiological hazard due to spoilage bacteria in frankfurters.

Estimation of shelf-life

The critical levels of microorganisms in meat products can vary. Thus, we followed the microbiological guidelines in the Korean Food Standards Codex (KFDA, 2008). The Korean Food Standards Codex confirms that the critical level of total viable cells in cooked meat products must be less than 4 Log CFU/g. Therefore, the shelf-life of frankfurters was determined based on the time required for total viable cells to reach a level of 4 Log CFU/g at each temperature condition (5, 15, and 25°C). The experimental data obtained were compared with each other (Table 5). The predicted shelf-lives were 10.5, 6.5,

Table 4. Developments and evaluation of secondary models of spoilage bacteria on frankfurters

Microorganisms	Polynomial model equation	Statistical analysis			
	$\text{Ln}(\mu_{max}) = a + bT + cT^2$	R^2 [1]	*RMSE* [2]	B_f [3]	A_f [4]
T [5]	$\text{Ln}(\mu_{max}) = 0.302 + 0.073 \cdot T - 0.001 \cdot T^2$	0.97	0.15	1.012	1.091
	$\text{Ln}(LT)) = 2.302 - 0.040 \cdot T - 0.001 \cdot T^2$	0.95	0.12	1.030	1.123
P [6]	$\text{Ln}(\mu_{max}) = -0.075 + 0.134 \cdot T - 0.003 \cdot T^2$	0.90	0.08	1.210	1.162
	$\text{Ln}(LT)) = 2.066 - 0.002 \cdot T - 0.002 \cdot T^2$	0.94	0.14	0.985	1.241
L [7]	- [8]	-	-	-	-
	-	-	-	-	-

[1] Correlation coefficient, [2] root mean square error, [3] bias factors, [4] accuracy factors, [5] total viable cells, [6] *Pseudomonas* spp., [7] lactic acid bacteria, [8] data not observed.

Table 5. Estimation of shelf-life of frankfurters at various temperatures (°C)

Microorganism	Temperature (°C)	Shelf-life (d)	
		Predicted shelf-life[1)]	Estimated shelf-life[2)]
Total viable cells	5	10.5	7.0
	15	6.5	4.3
	25	2.9	1.9

[1)] Days to reach 4 Log CFU/g, [2)] shelf-life calculated from Eq. (9).

and 2.9 (d) at 5, 15, and 25°C, respectively. As ambient temperatures increased, the number of total viable cells reached a level of 4 Log CFU/g more rapidly.

To determine frankfurter shelf-life more reliably, the predicted shelf-life was modified to the estimated shelf-life by including the safety factor (1/1.5). The estimated shelf-lives were therefore adjusted to 7.0, 4.3, and 1.9 (d). Thus, these results show that consumers can store frankfurters in the refrigerator (storing under 5°C) for 3 d more, even they buy frankfurters at the point of the predictive shelf-life (10.5 d). However, the gap between predictive and estimated shelf-life is dependant on the safety factor. The safety factor is changeable significantly depending on the characteristics of food and the regulation of the goverment. However, the information about the values of safety factor considering the characteristics of food is not enough to compare with our data yet. Therefore, further research needs to find the suitable safety factors, considering characteristics of food.

The interest in predictive modeling has increased remarkably in the food industry after the 1980s, and the usage of predictive modeling has induced renovation in the fields of product innovation, operational support, and incident support world wide (Membre and Lambert, 2008). By the application of predictive modeling, manufacturers can reliably estimate shelf-life and ensure the microbiological safety of foods. However, reliable standard methods for the estimation of shelf-lifes for various foods have not yet been developed in Korea Therefore, this research can contribute toward understanding predictive modeling and its application in the food industry.

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