



Models of *Pseudomonas* Growth Kinetics and Shelf Life in Chilled *Longissimus dorsi* Muscles of Beef*

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ABSTRACT : The aim of this study was to confirm *Pseudomonas spp.* as the specific spoilage organism (SSO) of chilled beef during aerobic storage and to establish a model to predict the shelf life of beef. Naturally contaminated beef was stored at 4°C, and the spoilage limit of *Pseudomonas* organisms was determined by measuring several quality indicators during storage, including the number of *Pseudomonas* organisms, total number of bacteria, total volatile basic nitrogen (TVBN) values, L value color scale scores and sensory evaluation scores. The beef was then stored at 0, 4, 7, 10, 15 or 20°C for varying amounts of time, and the number of *Pseudomonas* organisms were counted, allowing a corresponding growth model to be established. The results showed that the presence of *Pseudomonas spp.* was significantly correlated to each quality characteristic ($p < 0.01$), demonstrating that *Pseudomonas spp.* are the SSO of chilled beef and that the spoilage limit was $10^{8.20}$ cfu/g. The Baranyi and Roberts equation can predict the growth of *Pseudomonas spp.* in beef, and the R^2 value of each model was greater than 0.95. The square root model was used as follows, and the absolute values of the residuals were less than 0.05: $\mu_{\max}^{1/2} = 0.15604 [T + (-0.08472)]$ ($p < 0.01$), $R^2 = 0.98$, $\lambda^{-1/2} = 0.0649 + 0.0242T$ ($p < 0.01$, $R^2 = 0.94$). The model presented here describes the impact of different temperatures on the growth of *Pseudomonas spp.*, thereby establishing a model for the prediction of the shelf life of beef stored between 0 to 20°C. (**Key Words :** *Pseudomonas spp.*, Chilled Beef, Model, Shelf life)

INTRODUCTION

The environmental temperature, initial microbial contamination level and subsequent microbial growth and reproductions are important factors that affect the shelf life of meat products (Koutsoumanis et al., 2006; Brooks et al., 2008). To control food spoilage, it is necessary to understand the growth and reproduction patterns of the microorganisms that cause it and predict their growth at different temperatures (Miller et al., 1998). Microbiological predictions can do this quickly and effectively. Determining the product's specific spoilage organism (SSO) (Nychas, 2008) by using appropriate media for microbial growth

(Gill et al., 1997) and selecting effective mathematical equations (Van Impe et al., 2005) are the foundation for a good microbiological prediction model.

Pseudomonas has been studied as a model in fish (Koutsoumanis et al., 2001; Xu et al., 2005), chicken (Gospavic et al., 2008), pork (Fu et al., 2008) and beef (Jay et al., 2003; Koutsoumanis et al., 2006; Coll Cárdenas et al., 2008) although different modeling equations were used. Fu et al. (2008) used liquid media to establish a *Pseudomonas* growth model by fitting Gompertz equations while Jiang et al. (2008) inoculated *Pseudomonas* into sterile chilled pork and created a growth model with a Modified Gompertz equation. Gospavic et al. (2008) studied *Pseudomonas* in chicken and applied a Modified Gompertz equation and the Baranyi and Roberts equation simultaneously to establish a model; however, they did not formulate a shelf life prediction model. Koutsoumanis et al. (2006) studied the growth of four bacterial species in beef and pork under different conditions. They determined that *Pseudomonas* was the SSO of chilled beef due to its dominance and established a *Pseudomonas* growth model, but they did not give a detailed description or create a model to predict the

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remaining shelf life. The modeling equations used in these studies all differed, and the relationship between *Pseudomonas* and meat spoilage was not investigated: the studies only established that *Pseudomonas* was the SSO. Differences exist between naturally contaminated meat and artificially inoculated media, regardless of the type of media that is used. Using naturally contaminated meat in such studies can better reflect the growth of organisms and their relationship to spoilage. In the present study, the correlation between *Pseudomonas* and several indicators of spoilage were examined during the storage of chilled beef and confirmed that *Pseudomonas* is the SSO. The growth of *Pseudomonas* at 0, 4, 7, 10, 15 and 20°C were observed in naturally contaminated chilled beef, and the results fitted with the Baranyi and Roberts equation and established a model to predict the remaining shelf life. The results of this research will provide effective risk assessment tools and methods to accurately predict the remaining shelf life of beef.

MATERIALS AND METHODS

Determination of *Pseudomonas* as the SSO and of the minimum spoilage limit

Sample processing : Nine conventionally segmented cattle *longissimus dorsi* muscles were randomly selected from a cattle slaughter plant based in Shandong, China, and transported to the laboratory within 3 h between 0 to 4°C. The beef was randomly divided into three groups containing three pieces each. Tendons were removed aseptically, and the meat was cut perpendicular to the muscle fibers into 25 cm² pieces approximately 1 to 2 cm thick. The meat samples were packed in low-density storage bags and maintained at 4°C for 0, 2, 4, 6, 8, 10, 12 or 14 days. Every measurement was repeated at least 3 times.

Measurement of total bacteria and *Pseudomonas* : At designated time intervals, 25 g of meat from each sample group were placed in blender bags (BagFilter® 400, Interscience, France) and 225 ml of sterile peptone saline solution were added. Samples were processed for 60 s with a blender (BagMixer® Interscience, France) and serial diluted 10-fold to the desired dilution. Finally, 100 µl of samples from three dilutions were spread on culture plates in duplicate and the total number of bacteria and *Pseudomonas* organisms were determined. Samples were plated on regular nutrient agar and incubated aerobically at 37°C for 2 days to determine the total number of bacteria and on *Pseudomonas* medium with CFC (Oxoid, UK) and cultured aerobically at 25°C for 2 days to determine the number of *Pseudomonas* organisms.

Analysis of color differences : An X-Rite SP62 Portable Sphere Spectrophotometer was used to measure the CIE *L*a*b** color space of meat samples at various time points.

At least three sites were measured on each sample, and the average value and ΔE were calculated with the following equation, where *L*₀, *a*₀ and *b*₀ are the values measured on day 0 of storage, and *L*, *a* and *b* are the values measured at the corresponding time point during storage:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}$$

Measurement of total volatile basic nitrogen (TVBN) :

To measure the total volatile basic nitrogen (TVBN), 10 g of meat sample were minced, mixed well and placed in conical flasks. Then, 100 ml of water was added, and the suspension was occasionally agitated until they were filtered after 30 min of immersion. The filtrates were refrigerated until further use. The TVBN content was measured by the Method for analysis of hygienic standard of meat and meat products: GB/T5009.44-2003 (semi-micro diffusion method).

Sensory evaluation : Eight trained evaluators performed the sensory evaluation. Meat samples were evaluated based on color, odor, tissue status, viscosity, boiled broth and a comprehensive evaluation. A relatively simple seven-point method was applied: a score of one indicated the best quality while seven was the acceptable limit (Brooks et al., 2008). The sensory rejection point was defined as when half or more of the evaluators rated a sample at four points or above. The evaluation method was implemented according to Fresh and frozen beef, cuts: GB17238-2008.

Establishment and evaluation of models

Sample processing : As described in section-Determination of *Pseudomonas* as the SSO and of the minimum spoilage limit-Sample processing, each sample was packed in low-density storage bags and kept at 0, 4, 7, 10, 15 or 20°C. Three storage experiments were taken at different temperatures, and each treatment had three replications.

Determination of the number of *Pseudomonas* organisms : The number of *Pseudomonas* organisms were determined as described in section - Determination of *Pseudomonas* as the SSO and of the minimum spoilage limit-Measurement of total bacteria and *Pseudomonas*.

Establishment of a primary model for *Pseudomonas* growth kinetics : Experimental data from the meat samples stored at 0, 4, 7, 10, 15 and 20°C were applied to the Baranyi and Roberts equation (1) to describe the growth kinetics at different temperatures.

$$y(t) = y_{\max} + \ln\left(\frac{1 - \exp(-h) + \exp(\mu_0 t - h)}{\exp(y_{\max} - y_0 + \exp(\mu_0 t - h)) - \exp(-h)}\right) \quad (1)$$

$$\text{When, } y_{\max} \gg y_0, \mu_{\max} = \mu_0 \quad (2)$$

In equation (1), y_{\max} represents the maximum number of microbes as they reach the stationary phase (log cfu/g); y_0 is the initial number of microorganisms at $t = 0$ (log cfu/g); μ_0 is the specific growth rate (h^{-1}); and h is the adjusting factor. This equation is more accurate when h is fixed (Van Impe et al., 2005). In equation (2), μ_{\max} is the maximum specific growth rate (h^{-1}).

A secondary model to predict the impact of temperature on Pseudomonas growth kinetics : Ratkowsky et al. (1982) proposed a square root equation for an empirical model based on the linear relationship between the temperature and the square root of microorganisms' growth rate or the square root of the reciprocal of the lag phase under different temperature conditions:

$$\sqrt{1-\lambda} = b_{\lambda} \times (T - T_{\min\lambda}) \quad (3)$$

$$\sqrt{\mu_{\max}} = b_{\mu} \times (T - T_{\min\mu}) \quad (4)$$

In equations (3) and (4), T is the temperature ($^{\circ}C$), T_{\min} refers to the hypothetical temperature at which no microbial metabolic activity occurs, or the temperature at which the maximum specific growth.

Validation of Pseudomonas growth kinetics model and reliability assessment : The predicted values for storage at 4 and $10^{\circ}C$ were obtained using the established microbial growth kinetics model and compared to the experimentally measured growth in meats stored at 4 and $10^{\circ}C$ from another cattle slaughter plant. The reliability of the established growth kinetics model of the SSO was evaluated by using the bias factor (B_f) and the accuracy factor (A_f).

$$B_f = 10^{[\sum(N_{\text{predicted}} - N_{\text{actual}})]/n} \quad (5)$$

$$A_f = 10^{[\sum|N_{\text{predicted}} - N_{\text{actual}}|]/n} \quad (6)$$

In equations (5) and (6), N_{actual} is the number of the actual microorganisms in the sample; $N_{\text{predicted}}$ is the number of microorganisms predicted by the microbial growth kinetics model at the same time point as N_{actual} ; and n is the number of experiments.

Shelf life prediction and verification : The shelf life (SL) of chilled beef can be predicted using the established growth kinetics model for the SSO by calculating the proliferation time necessary to increase from the initial number of the SSO (N_0) to the spoilage limit number (N_s). In equation (7), λ is the lag time for microbial growth (h), and μ_{\max} is the maximum specific growth rate (h^{-1}).

$$SL = \lambda - [(N_{\max} - N_0) / \mu_{\max} \times 2.718] \times \{ \ln [-\ln[(N_s - N_0) / (N_{\max} - N_0)] - 1] \} \quad (7)$$

The reliability of the shelf life prediction model was evaluated by comparing the actual shelf life determined from storage experiments at 4 and $10^{\circ}C$ with shelf life predicted from the model.

Data processing

Data were analyzed using the statistical software package SAS 9.0 for variance, correlation and cluster analyses. An appropriate model was selected for the fitting and regression analyses of the experimental data for the shelf life prediction using the least squares method.

RESULTS AND ANALYSES

Identification of Pseudomonas as the SSO and determination of the minimum limit of spoilage

Correlation analysis of Pseudomonas and evaluation of quality characteristics : The correlation coefficient of the number of *Pseudomonas* and the TVBN is 0.89335 ($p < 0.01$) in beef stored at $4^{\circ}C$ (Table 1), suggesting a high correlation. TVBN is a result of protein decomposition due to the decarboxylation and deamination caused by the growth of microorganisms, including *Pseudomonas*. It is an important meat spoilage indicator as nitrogenous compounds, including primary, secondary, tertiary amines and others, are released. The correlation coefficient of the *Pseudomonas* number and color scale L value was -0.93351 ($p < 0.01$), suggesting that they are also highly correlated. While the color of meat products is not only the criteria a consumer considers when choosing a product (Mancini and Hunt, 2005), it is an important indicator of meat quality, hygiene and freshness. The color depends not only on the meat's myoglobin and hemoglobin content but also on the chemical states of myoglobin and hemoglobin (Mancini and Hunt, 2005). The growth of *Pseudomonas* constantly reduces the partial pressure of oxygen and gradually promotes the formation of metmyoglobin (metMb), resulting in a gradual change in meat color. The present study used a seven-point method to comprehensively evaluate the degree of meat spoilage using the beef's color, odor, flexibility, tissue status, viscosity and boiled broth as quality characteristics. The correlation coefficient of the sensory evaluation scores and *Pseudomonas* was 0.97033 ($p < 0.01$).

To determine the SSO for a product, two requirements must be met: first and most important, it must be the dominant organism over a prolonged period, and second, it must be highly correlated with spoilage of the product (Nychas et al., 2008). Because *Pseudomonas* has been widely reported as the dominant bacteria in beef stored at

Table 1. Pearson correlation coefficients of the quality characteristics of beef stored at 4°C

	Number of <i>Pseudomonas</i> organisms	Total number of colonies	TVBN	Sensory evaluation value	L*	a*	b*	ΔE
Number of <i>Pseudomonas</i> organisms	1.0000	0.94475**	0.89335**	0.97033**	-0.92555**	-0.78494*	-0.76456*	0.73140*
Total number of colonies		1.00000	0.83427**	0.96276**	-0.88311**	-0.74248*	-0.62739*	0.70523
TVBN			1.00000	0.93571**	-0.93689**	-0.84939**	-0.82606*	0.87255**
Sensory evaluation value				1.00000	-0.93838**	-0.84707**	-0.75096*	0.84609**
L*					1.00000	0.80062**	0.83104*	-0.77744*
a*						1.00000	0.87346**	-0.88740**
b*							1.00000	-0.73674*
ΔE								1.00000

** Correlation is significant at the p = 0.01 level. * Correlation is significant at the p = 0.05 level.

low temperatures (Skandamis et al., 2002; Jay et al., 2003; Ercolini et al., 2006; Koutsoumanis et al., 2006), these experiments were not repeated. *Pseudomonas* growth is significantly correlated with the score of several quality indicators in beef stored at 4°C, such as the total number of bacteria, sensory evaluation scores, TVBN and color scale L values. Moreover, the correlation coefficients between *Pseudomonas* and each spoilage criteria were all greater than those between the total number of bacterial colonies and the spoilage criteria. Thus, it is concluded that *Pseudomonas* is the SSO, and it is practical to use a growth model to predict the degree of spoilage in aerobically stored chilled beef.

Determination of the *Pseudomonas* spoilage limit : In general, it is important to evaluate specific quality

characteristics and their corresponding critical thresholds to determine the shelf life of a product. A product will not be of acceptable quality if a specific characteristic surpasses its critical threshold (Brooks et al., 2008). In addition, the number and types of microorganisms must be considered when determining the shelf life of a product (Brooks et al., 2008). In this study, the number of *Pseudomonas* organisms, total number of bacteria, TVBN value, color scale L value and sensory evaluation scores were evaluated to judge the quality of chilled beef.

As shown in Table 2, the number of *Pseudomonas* organisms and the total number of bacteria increased with time. The increase was relatively large during the first eight days, corresponding to the lag and exponential growth phases. After eight days, the bacterial growth rate was fairly

Table 2. Quality characteristics of beef stored at 4°C (Mean±SD)

Characteristic \ Time (d)	Number of <i>Pseudomonas</i> (log ₁₀ cfu/g)	Total number of bacteria (log ₁₀ cfu/g)	TVBN value (mg/100 g)	Color scale L value	Sensory evaluation score
0	2.854 ^{b*} ±0.077	4.987 ^b ±0.184	10.196 ^a ±0.393	39.652 ^a ±1.387	1.709 ^a ±0.646
1	2.327 ^a ±0.213				
2	2.746 ^a ±0.131	4.511 ^a ±0.028	11.096 ^{ab} ±0.836	40.132 ^a ±3.363	1.873 ^{ab} ±0.681
3	4.127 ^c ±0.023				
4	5.053 ^d ±0.005	6.145 ^c ±0.002	10.924 ^{ab} ±0.340	39.852 ^a ±1.097	2.237 ^b ±0.682
5	6.117 ^e ±0.107				
6	6.163 ^e ±0.002	6.312 ^c ±0.025	12.167 ^b ±0.594	38.348 ^{ab} ±1.640	2.691 ^{bc} ±0.757
7	7.292 ^f ±0.003				
8	8.169 ^g ±0.002	8.143 ^d ±0.071	11.567 ^b ±0.463	38.197 ^b ±0.782	3.077 ^c ±0.760
9	8.863 ^g ±0.008				
10	8.603 ^h ±0.084	8.6417 ^e ±0.056	21.034 ^c ±0.487	36.585 ^c ±1.010	3.924 ^d ±0.839
12	8.744 ^{hi} ±0.017	8.662 ^e ±0.100	23.905 ^d ±0.564	34.422 ^d ±2.542	4.372 ^e ±0.691
14	10.265 ⁱ ±0.003	9.356 ^f ±0.171	25.490 ^e ±0.753	35.33 ^{cd} ±1.190	5.027 ^f ±1.086

Different letters in the same row indicate a significant difference (p<0.05).

steady. The number of *Pseudomonas* organisms and total bacteria at day 8 were significantly different from those at day 10. The TVBN value during the first eight days of storage showed little change and was steady around 10 mg/100 g. However, on day 10, the value was 21.03 mg/100 g, greater than the Hygienic standard (for fresh (frozen) meat of livestock: GB2707-2005 (TVBN value ≤ 15 mg/100 g) and was significantly different from the values obtained in the first eight days. The L value describes the brightness in the meat, and this L value indicated that the meat color did not change much during the initial storage period with a value around 39. However, starting at day 8, the brightness values plummeted and the value at day 10 was significantly different from those recorded in the first eight days. The sensory evaluation scores at day 10 were near 4 points, which is considered to be unacceptable and indicates that by this point, the meat had spoiled.

Additionally, various characteristics were analyzed in cluster analyses. Samples from the first eight days clustered in a single group whereas those stored for longer than eight days grouped separately (Figure 1). When considering the total number of bacteria, sensory evaluation scores and TVBN values, the results concluded that the products are acceptable for consumption for the first eight days of storage, but that products stored for greater than eight days spoil and are unacceptable. The presence of *Pseudomonas* is significantly correlated with each spoilage characteristic, and the spoilage limit is set at $10^{8.2}$ cfu/g in chilled beef. When the number of *Pseudomonas* exceeds this value, the meat is considered to be spoiled. This result is consistent with the conclusions of Koutsoumanis et al. when they determined that beef is no longer acceptable for consumption when the number of *Pseudomonas* organisms

reaches 10^7 to 10^9 cfu/g (Koutsoumanis et al., 2006).

Model development and validation

Primary model fitting : Based on the number of *Pseudomonas* organisms determined under constant temperature conditions, different primary models were fitted and Time-log cfu/g curves at different temperatures were drawn (Figure 2). The corresponding fitting equations are ($h = 1.5517$):

$$0^{\circ}\text{C: ypb00} \\ = 8.6263 + \ln(0.78811 + \exp(0.0299 * t - 1.5517)) \\ / (381.572 + \exp(0.0299 * t - 1.5517) - 0.21189);$$

$$4^{\circ}\text{C: ypb04} \\ = 9.2125 + \ln(0.78811 + \exp(0.0386 * t - 1.5517)) \\ / (279.807 + \exp(0.0386 * t - 1.5517) - 0.21189);$$

$$7^{\circ}\text{C: ypb07} \\ = 8.9673 + \ln((0.78811 + \exp(0.0525 * t - 1.5517)) \\ / (189.426 + \exp(0.0525 * t - 1.5517) - 0.21189));$$

$$10^{\circ}\text{C: ypb10} \\ = 8.8588 + \ln((0.78811 + \exp(0.0896 * t - 1.5517)) \\ / (213.92 + \exp(0.0896 * t - 1.5517) - 0.21189));$$

$$15^{\circ}\text{C: ypb15} \\ = 8.9794 + \ln((0.78811 + \exp(0.1259 * t - 1.5517)) \\ / (205.183 + \exp(0.1259 * t - 1.5517) - 0.21189));$$

$$20^{\circ}\text{C: ypb20} \\ = 8.7884 + \ln((0.78811 + \exp(0.1795 * t - 1.5517)) \\ / (146.995 + \exp(0.1795 * t - 1.5517) - 0.21189));$$

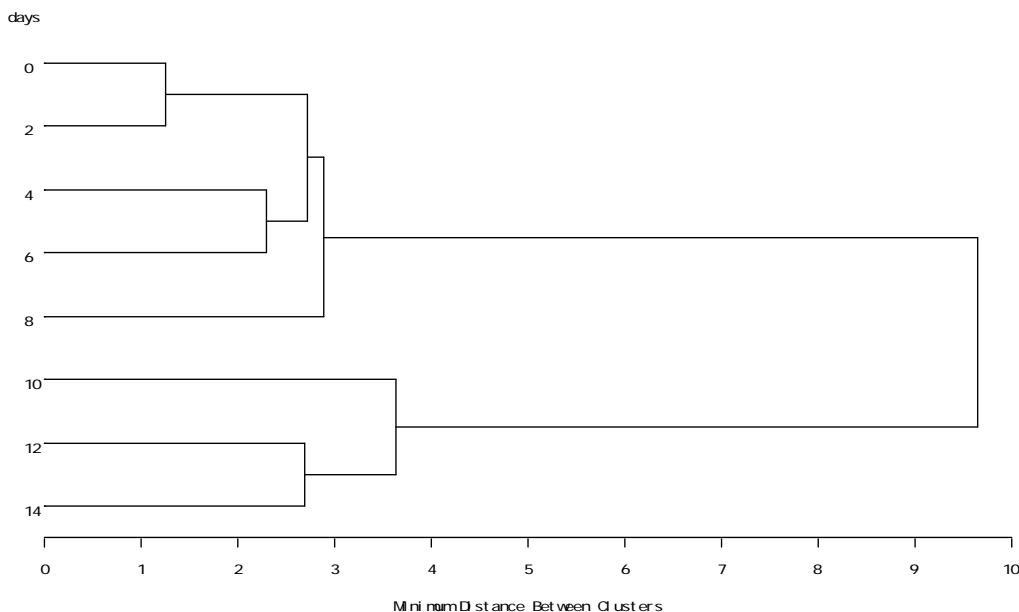


Figure 1. Dendrogram using single linkage according to the quality indicators of beef during the storage days (between groups).

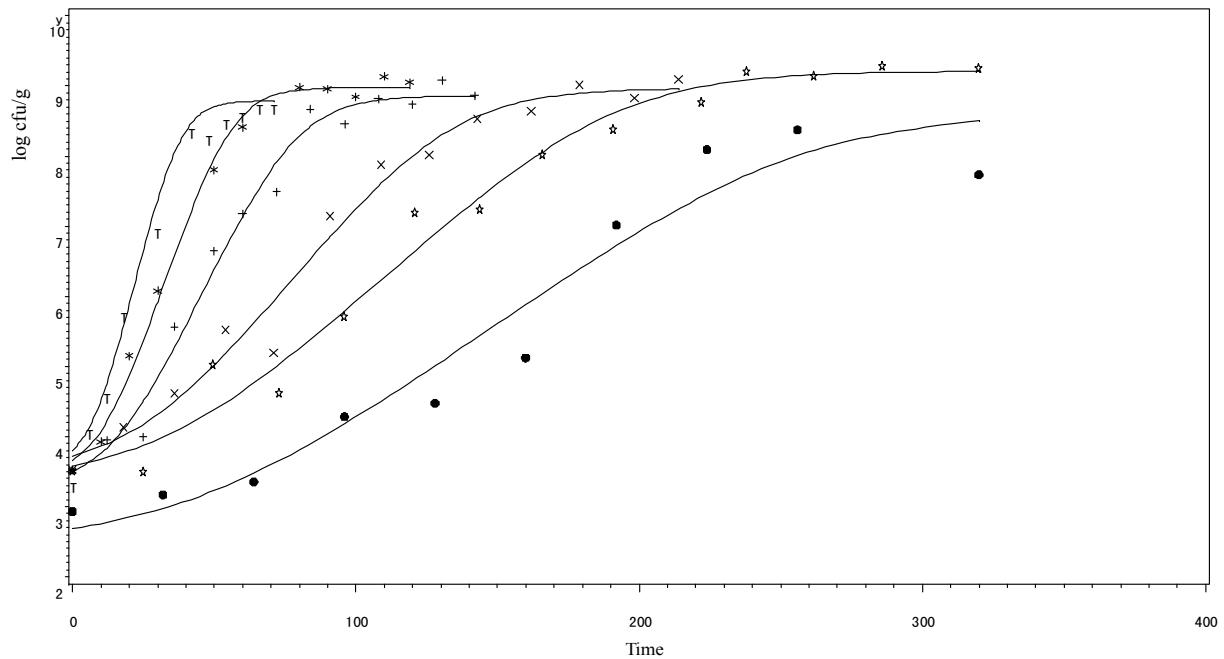


Figure 2. Growth data and the fitted Baranyi and Roberts curves of the growth of *Pseudomonas spp.* on aerobically stored beef at 0, 4, 7, 10, 15 and 20°C. (Note: The fitted curves are 0, 4, 7, 10, 15 and 20°C from right to left; Different symbols (●, ☆, ×, +, *, T) represent the actual values at 0, 4, 7, 10, 15 and 20°C respectively).

As shown in Figure 2, this model fits well with the growth of *Pseudomonas* at different temperatures. The growth curve is a typical S-type and microbial growth was significantly affected by temperature. As the temperature increased, the amount of time needed to reach the maximum number of bacteria decreased; approximately 320 hours were needed at 0°C but only 70 hours were necessary at 20°C. The different parameters included in the model include the initial number of microorganisms, maximum number of bacteria, maximum specific growth rate and lag phase growth rate. The mean square error (MSE) and R^2 values are listed in Table 3.

The impact of temperature on the growth of *Pseudomonas* is detailed in Table 3. The specific growth rate in the model increased with rising temperatures: it was 0.03 h^{-1} at 0°C, was 0.0896 h^{-1} at 10°C, and at 20°C the

specific growth rate increased by a factor of 6 to 0.18 h^{-1} . The time at which lag phase was reached decreased from 104.36 h at 0°C to 2.74 h at 20°C, which is different from the observation of Cárdenas et al. (2008) where they found that the specific growth rate was not significantly different at 4 and 10°C. However, the present results were similar to those obtained in a study by Giannuzzi et al. (1998), which showed that temperature is an important factor in microbial growth. If the temperature is too low, the plasma membrane freeze and be unable to transport nutrients or form a proton gradient; thus, growth is not possible. When the temperature increases to the organisms' growth temperature range (0 to 25°C), the intracellular enzymatic reactions can be performed, the metabolic rate increases and the growth rate is accelerated (Huang et al., 2009). Therefore, the specific growth rate of *Pseudomonas spp.* between 0 to 20°C

Table 3. Initial number of *Pseudomonas* organisms (N_0), maximum number of bacteria (N_{\max}), Maximum specific growth rate (μ_{\max}), lag phase (λ), mean square error (MSE), and R^2 obtained by the Baranyi and Roberts equation in chilled beef stored at 0, 4, 7, 10, 15 and 20°C

Temperature (°C)	N_0 (\log_{10} cfu/g)	N_{\max} (\log_{10} cfu/g)	μ_{\max} (h^{-1})	λ (h)	MSE	R^2
0	2.6820±0.3134	8.6263±0.3254	0.0299±0.0027	104.3628	0.2772	0.9583
4	3.5784±0.1986	9.2125±0.1671	0.0386±0.00229	26.3971	0.1107	0.9803
7	3.7233±0.1916	8.9673±0.07104	0.0525±0.00326	25.0739	0.0998	0.9801
10	3.4932±0.1784	8.8588±0.1327	0.0896±0.00494	16.6048	0.0783	0.9849
15	3.6555±0.1191	8.9794±0.0786	0.1259±0.00531	6.1344	0.0309	0.9948
20	3.7980±0.1333	8.7884±0.0941	0.1795±0.00963	2.7448	0.0411	0.9924

increases and the time to reach lag phase decreases. The best fitting model will have the smallest possible p and MSE values and a maximized R^2 . Because the p value in both models were smaller than 0.0001, the R^2 values were greater than 0.95, and the MSEs were relatively small, both models have been determined to fit well to the experimental data. The maximum number of *Pseudomonas* organisms at each temperature was calculated, with an average of $10^{8.91}$ cfu/g (Table 3).

The results presented in Table 3 are somewhat contradictory with results from a study by Fu et al. (2008) and Miller (1998), in which liquid medium was used. Cultivation of a single bacterial strain under standard laboratory conditions does not mimic conditions in an actual meat sample with the interaction of several bacterial species. Thus, inconsistencies between these results are expected. Moreover, more than one bacterial species can contribute to beef spoilage (for example, *Pseudomonas* includes at least three strains (Ercolini et al., 2007), and identification of the type and number of bacteria using a microbial predictive model established with artificially inoculated samples is difficult regardless of the culture method used. Therefore, using naturally contaminated beef to establish a model will produce more consistent and realistic results and provide a more practical model.

Secondary model fitting: A square root model is mainly used to describe the impact of environmental factors. In this method, the simple expression describing the impact of temperature is: $U = b(T - T_{\min})$, where b is the coefficient [$d^{1/2} \text{ } ^\circ\text{C}^{-1}$], and T_{\min} is the theoretical lowest temperature for microbial growth. SAS statistical software fit the maximum specific growth rate/temperature curve ($\mu_{\max}^{1/2} - T$) using the present experimental *Pseudomonas* growth rates at different temperatures (Table 3) to produce the secondary model, $\mu_{\max}^{1/2} = 0.01322 + 0.15604T$ ($p < 0.01$, $R^2 = 0.98$), or: $\mu_{\max}^{1/2} = 0.15604[T + (-0.08472)]$. By replacing the variables with the biologically relevant parameters, parameters that for *Pseudomonas* were determined, $b = 0.15604 d^{-1/2} \text{ } ^\circ\text{C}^{-1}$, and $T_{\min} = -0.08472 \text{ } ^\circ\text{C}$. The time to lag phase in *Pseudomonas* was $\lambda^{-1/2} = 0.0649 + 0.0242T$ ($p < 0.01$, $R^2 = 0.936$).

The square root model is the most commonly studied model and is extensively used to fit the relationship between specific growth rates and time. Table 4 lists the temperature residuals and specific growth rates in the square root model. The temperature residuals were compared and analyzed using the observed and predicted values, and their absolute values were all less than 0.05 (Table 4), indicating that the relationship between temperature and the maximum specific growth rate described by this model is reliable. Thus, this model can describe the impact of different temperatures on microbial growth.

Model verification: Bias factors serve as a measurement

Table 4. Temperature residuals and $\mu_{\max}^{1/2}$

Temperature ($^\circ\text{C}$)	Observed value	Predicted value	Residuals
0	0.0299	0.024348	0.01687
4	0.0386	0.043643	-0.01244
7	0.0525	0.061777	-0.01942
10	0.0896	0.083059	0.0111
15	0.1259	0.125514	0.000543
20	0.1795	0.176686	0.00331

index for the average variation between the predicted and observed values. The accuracy factor is a measurement to estimate the accuracy of an established model. In this study, a large accuracy factor indicates inaccurate results, and a value of 1 is the ideal result. Table 5 presents the bias factors (B_f) and accuracy factors (A_f) from studies at 4 and 10°C . The accuracy factor was calculated using the predicted and actual values of samples during storage in different slaughter facilities. Predicted values fluctuate by about 10%, and the difference between the predicted and observed values (A_f) is within 11%, indicating that the error is relatively low. Thus, the established model can predict the growth of SSOs at 4 and 10°C .

Prediction of remaining shelf life: According to the model of *Pseudomonas* growth kinetics in the present research, the remaining shelf life of beef stored aerobically at $0\text{--}20^\circ\text{C}$ can be predicted by calculating the time required for *Pseudomonas* organisms to increase from their initial population count (N_0) to the minimum limit of spoilage (N_s). The N_s for *Pseudomonas* is $10^{8.20}$ cfu/g, and the maximum number of bacteria (N_{\max}) is $10^{8.91}$ cfu/g. Therefore, if the temperature and initial number of *Pseudomonas* organisms (N_0) is accurately known, the remaining shelf life of beef stored between 0 and 20°C can be calculated based on the following formula and second order equation using equation (7):

$$SL = \lambda - [(8.20 - N_0) / \mu \times 2.718] \\ \times \{ \ln [-\ln [(8.20 - N_0) / (8.91 - N_0)] - 1] \}$$

Li et al. (2008) used naturally contaminated samples to determine the spoilage limit, but used artificially inoculated samples to establish their shelf life prediction model. All

Table 5. Bias and accuracy factors of predicted values of microbial counts for beef stored at 4 and 10°C

Storage temperature ($^\circ\text{C}$)	Test number (n)	Bias factor (B_f)	Accuracy factor (A_f)
4	5	0.988517	1.080675
10	6	1.031562	1.108038

samples used in this study to determine the spoilage limit and establish a prediction model were naturally contaminated and obtained from the normal production line. Thus, they are more similar to meat prepared for consumption, and our prediction model has a greater practical value to guide the actual meat production business.

DISCUSSION

Pseudomonas spp. play a dominant role particularly due to their ability to use glucose from the meat in the ED pathway to produce gluconic acid and 2-oxogluconate under aerobic conditions. These compounds accumulate outside of the cells and are further utilized by *Pseudomonas* while competing bacteria are unable to use them. When the number of *Pseudomonas* organisms reaches $10^{8.0}/\text{cm}^2$, the glucose supply can no longer meet their growth needs. At this point, they begin to use amino acids to facilitate growth and generate sulfur-containing compounds, esters and acids, among others. In addition, *Pseudomonas* is the predominant microorganism causing meat spoilage because it has a greater ability to utilize the glucose and amino acids at lower temperatures than other bacteria (Ercolini et al., 2006). The decomposition of glucose and amino acids has direct or indirect effects on the TVBN and color values of the meat, leading to eventual spoilage. The effects of the interactions between different microorganisms causing meat spoilage should be further studied.

The value of pH, color scale a and b values, ΔE and the meat shear force values were also studied as possible indicators of spoilage, but the correlations of these values with the number of *Pseudomonas* organisms were not significant (data not shown). Further studies are needed to determine whether these characteristics are indicative of spoilage in chilled beef. In addition, some biogenic amines have been described as meat spoilage indicators (Li et al., 2006) but the feasibility of these studies in chilled beef requires further verification.

The most commonly used primary models include the Gompertz equation, the Baranyi and Roberts equation and the logistic model (McDonald et al., 1999), among others. A Gompertz equation modified by Zwietering (the Modified Gompertz equation) can more accurately describe growth dynamics at different temperatures (Dalgaard et al., 1995). Experimental results verified that the logistic model does not work as well as the modified Gompertz equation at low temperatures (Fu, 2008); therefore, the modified Gompertz equation and the Baranyi and Roberts equation are the most widely used primary models. Both models have advantages and disadvantages, and there is no conclusion as to which is more practical. In this study, the two models worked well in the primary model (modified Gompertz equation results not shown), but in the secondary model, the results of the

specific growth rates obtained by the Baranyi and Roberts equation had significantly smaller confidence intervals than the modified Gompertz equation. Furthermore, analysis of variance showed that the p, MSE and R^2 values from the Baranyi and Roberts model had better values than the other, which to some extent supports that the Baranyi and Roberts equation works better than the modified Gompertz equation in this application. However, a thorough comparison of the models requires a significant amount of experimental data.

The experimental data in this study was collected from naturally contaminated beef, while many other studies used liquid media to model microbial growth, which is not as accurate. Liquid media does not mimic the actual conditions in meat because the types of meat tissue and interactions between microorganisms have a large impact on the growth of different microbes. Differences in the slaughter plant procedures and transportation may also play a role in the composition of the microbial flora of the meat (Gill et al., 2000). The impacts of the type of muscle tissue and interactions between microorganisms on the growth of *Pseudomonas* have been considered in our model. Our study reflects the natural growth process of the microorganisms, and to a certain extent, this improves the accuracy of our prediction model compared to others. This study provides an effective tool for predicting and monitoring the quality and freshness of chilled beef. However, the actual temperature of meat products during storage and transportation often fluctuates, and it is important to accurately estimate the effects of temperature changes on the shelf life of the product (Tsironi et al., 2009). Therefore, it is necessary to further study this prediction model at fluctuating temperatures.

CONCLUSIONS

The correlation between the growth of *Pseudomonas* and the total number of bacteria, TVBN value, color scale L value, sensory evaluation and other quality indicators is statistically significant ($p < 0.01$); therefore, *Pseudomonas* is considered to be the SSO for chilled beef. It is feasible to use a *Pseudomonas* growth model to predict the extent of spoilage of chilled beef under aerobic storage conditions. Cluster analysis and the observations of the aforementioned quality indicators determined that the spoilage limit of *Pseudomonas* in chilled beef is $10^{8.20}$ cfu/g.

SAS statistical software was used to analyze *Pseudomonas* growth at different temperatures. The Baranyi and Roberts equation was a good predictor of *Pseudomonas* growth in the beef; the growth curve was a typical S-type, and the p values were less than 0.0001, MSE values were less than 0.3 and R^2 values were greater than 0.95. The square root model was used to consider the maximum specific growth rate and lag phase of *Pseudomonas* and

temperature ($\mu_{\max}^{1/2}-T$; $\lambda^{-1/2}-T$), and secondary models of *Pseudomonas* growth were obtained: $\mu_{\max}^{1/2} = 0.15604[T + (-0.08472)]$, $\lambda^{-1/2} = 0.0649 + 0.0242T$ ($p < 0.01$, $R^2 = 0.94$). By comparing the residuals of observed values with those of the predicted values, the absolute values were less than 0.05, indicating that the square root model can accurately describe the effect of different temperatures on microbial growth.

Chilled beef samples were obtained from several slaughter plants, and the number of *Pseudomonas* organisms at 4 and 10°C were counted to verify our model. The predicted values fluctuated in a range of about 10%, and the differences between predicted and observed values (A_f) were less than 11%. The established secondary model using the Baranyi and Roberts equation predicted the growth of *Pseudomonas* in chilled beef, and it is a good foundation for further study on the suitability of this prediction model at fluctuating temperatures and to develop software to carry out these models. This study provides an equation to predict the shelf life of chilled beef provided that the initial number of *Pseudomonas* organisms (N_0) and the actual temperature are known.

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