pISSN 1229-1153 *J. Fd Hyg. Safety*
Vol. 28, No. 3, pp. 217~221 (2013)
http://dx.doi.org/10.13103/JFHS.2013.28.3.217

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

Mathematical Models to Predict *Staphylococcus aureus* Growth on Processed Cheeses

Kyungmi Kim, Heeyoung Lee, Jinsan Moon[1], Youngjo Kim[2], Eunjeong Heo[3], Hyunjung Park[4], and Yohan Yoon*

Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, Korea
[1]Veterinary Pharmaceutical Management Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang 430-824, Korea
[2]Livestock Products Sanitation Division, Ministry of Food and Drug Safety, Osong 363-700, Korea
[3]Food Microbiology Division, Ministry of Food and Drug Safety, Osong 363-700, Korea
[4]Agro-Livestock and Fishery Products Policy Division, Ministry of Food and Drug Safety, Osong 363-700, Korea
(Received June 20, 2013/Revised July 24, 2013/Accepted August 14, 2013)

ABSTRACT - This study developed predictive models for the kinetic behavior of *Staphylococcus aureus* on processed cheeses. Mozzarella slice cheese and cheddar slice cheese were inoculated with 0.1 ml of a *S. aureus* strain mixture (ATCC13565, ATCC14458, ATCC23235, ATCC27664, and NCCP10826). The inoculated samples were then stored at 4°C (1440 h), 15°C (288 h), 25°C (72 h), and 30°C (48 h), and the growth of all bacteria and of *S. aureus* were enumerated on tryptic soy agar and mannitol salt agar, respectively. The Baranyi model was fitted to the growth data of *S. aureus* to calculate growth rate (μ_{max}; log CFU·g^{-1}·h^{-1}), lag phase duration (LPD; h), lower asymptote (log CFU/g), and upper asymptote (log CFU/g). The growth parameters were further analyzed using the square root model as a function of temperature. The model performance was validated with observed data, and the root mean square error (RMSE) was calculated. At 4°C, *S. aureus* cell growth was not observed on either processed cheese, but *S. aureus* growth on the mozzarella and cheddar cheeses was observed at 15°C, 25°C, and 30°C. The μ_{max} values increased, but LPD values decreased as storage temperature increased. In addition, the developed models showed acceptable performance (RMSE = 0.3500-0.5344). This result indicates that the developed kinetic model should be useful in describing the growth pattern of *S. aureus* in processed cheeses.

Key words: *Staphylococcus aureus*, processed cheese, predictive model

Introduction

The consumption of cheese has increased globally, and in particular, in Korea, since the 1990s[1]. Cheese is regarded as one of the safest foods, but it is often implicated in food-borne outbreaks, especially of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*[2,3]. In general, there are many outbreaks of *S. aureus*[4]. The bacterium produces enterotoxins, causing intoxication and food-borne illness[5]. *S. aureus* usually exists on the skin and nasal passages, and the pathogen can easily contaminate food[6,7]. The foods commonly associated with staphylococcal intoxication are meat products, salads, cream-filled bakery products, and dairy products[8]. To describe kinetic behavior of *S. aureus* in the foods, many mathematical models have been thus developed. However, there have been reports from many countries about *S. aureus* being isolated from various cheese products[9,10]. Hence, many countries have a quantitative standard or "zero tolerance" policy to control the pathogens growing on cheese[11,12,13,14,15]. Furthermore, mathematical models need to be developed to describe kinetic behavior of *S. aureus* in cheese, which should be useful in exposure assessment.

Predictive microbiology has been used to develop mathematical models to predict the response of food-borne pathogens to food-related environments[16,17]. Usually, mathematical models have been used for exposure assessment in microbial risk assessment[18]. A primary model describes the kinetic behavior of food-borne pathogens on foods, and a secondary model describes environmental influences on kinetic parameters such as maximum specific growth rate (μ_{max}) and lag phase duration (LPD)[19]. Therefore, the objective of this study was to develop mathematical models to describe the kinetic behavior of *S. aureus* on cheese.

*Correspondence to: Yohan Yoon, Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, Korea
Tel: 82-2-2077-7585, Fax: 82-2-710-9479
E-mail: yyoon@sookmyung.ac.kr

Materials and Methods

Inoculum preparation

Colonies of *S. aureus* strains ATCC13565, ATCC14458, ATCC23235, ATCC27664, and NCCP10826 cultured on mannitol salt agar (MSA; BBL™, Becton Dickinson and Company, Sparks, MD, USA) were inoculated into 10 ml tryptic soy broth (TSB; Bacto™, Becton Dickinson and Company, Sparks, MD, USA), and incubated at 35°C for 2 h. An aliquot (0.1 ml) of each culture suspension was inoculated into 10 ml TSB for subculturing at 35°C for 24 h. The cultures were then mixed and centrifuged at 1,912 × *g* and 4°C for 15 min. The resulting pellet was thoroughly washed with phosphate-buffered saline (PBS; pH 7.4, KH_2PO_4 0.2 g, Na_2HPO_4 1.5 g, NaCl 8.0 g, KCl 0.2 g per liter of distilled water) twice. The pellet was resuspended in PBS, and the cell suspension was then diluted with PBS to 5-6 log CFU/ml.

Sample preparation and inoculation

Two commercial processed cheeses (mozzarella and cheddar slice cheese) were used in this study. Each 18-gram formed slice of cheese was removed from its plastic wrapping and inoculated by spreading 0.1 ml of the inoculum on its surface by using a sterile bent spreader. The samples were then returned to their original plastic wrapping, and two samples were sealed in a plastic bag by a sealer (Food Guard®; Rollpack, Pyeongtaek, Gyeonggi-do, Korea). The sealed samples were then stored at 4°C, 15°C, 25°C, and 30°C for 1440 h, 288 h, 72 h, and 48 h, respectively.

Analyses

To enumerate bacterial populations, the cheese samples were transferred to a filter bag (BagFilter®, Interscience, St. Nom, France) containing 30 ml 0.1% buffered peptone water (BPW; Difco™, Becton Dickinson and Company, Sparks, MD, USA). The bagged samples were homogenized in a pummeler (BagMixer®, Interscience, St. Nom, France) for 2 min, and the homogenates were then serially diluted with BPW. Aliquots (0.1 ml) of the diluted homogenates were surface-plated onto tryptic soy agar (Difco™) and MSA for determining total bacterial counts and *S. aureus* counts, respectively. The plates were incubated at 35°C for 24 h, and the colonies were manually counted. The pH of the homogenates was measured with a digital pH meter (Accument®, Denver Instruments, Arvada, CO, USA). The water activity (a_w) of the samples was measured with a water activity meter (Aquaspector®, NAGY Messsysteme GmbH, Germany).

Primary model

The procedure was performed twice with two replicates of each sample (n = 4). The Baranyi model[20] was fitted to the *S. aureus* growth data for each cheese product and the kinetic parameters such as the maximum specific growth rate (μ_{max}; log CFU·g^{-1}·h^{-1}) and LPD (h) were calculated with the DMFit package (Institute of Food Research, Norwich, UK).

Secondary model

The square root model was fitted to μ_{max} and LPD values as a function of temperature to evaluate the effect of storage temperature on the kinetic parameters. The square root model and exponential decay model was suitable for fitting of μ_{max} and LPD, respectively. The function of these models are as follows

$$\sqrt{\mu_{max}} = \alpha(T - T_{min}) \quad (1)$$

Where α is the slopes of linear regression, T is storage temperature, and T_{min} is the theoretical minimum temperature value for the growth of *S. aureus* on processed cheese.

$$\text{LPD} = a \times e^{-b \times T} \quad (2)$$

Where a is the initial value, and b is the increase rate.

Validation

To validate the developed models, observed *S. aureus* cell counts were obtained from another study. These observed data were then compared with the predicted *S. aureus* cell counts, which were calculated using the models developed. Subsequently, the root mean square error (RMSE) was calculated to evaluate the model performance, as follows:

$$\text{RMSE} = \sqrt{\frac{\sum(\text{observed values} - \text{predicted values})^2}{n-1}} \quad (3)$$

In the above equation, "n" represents the number of observations.

Statistical analysis

Growth parameters were analyzed by the general linear model procedure of SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA), and the LS means among temperatures were compared with pairwise *t*-test at alpha = 0.05.

Results and Discussion

No bacterial growth was observed at 4°C from samples from the mozzarella or the cheddar slice cheese (data not shown). Thus, primary models were developed using only the *S. aureus* growth data from the 15°C, 25°C, and 30°C. No growth at 4°C may be caused by lowered affinity for substrates because low temperature may decrese the active transport system[21]. For both cheeses, the LPD values of *S.*

aureus decreased from 3.10 to 48.00 h (mozzarella slice cheese) and from 0.08 to 130.01 h (cheddar slice cheese) as the storage temperature increased (Table 1). At 15°C, LPD for the cheddar slice cheese (130.01 h) was longer ($P < 0.05$) than that of the mozzarella slice cheese (48 h) (Table 1).

S. aureus cell counts from the mozzarella slice cheese gradually increased to 8.4 log CFU/g at 15°C. However, *S. aureus* cell counts on the cheddar cheese increased only to 4.9 log CFU/g at this temperature (Table 1). At 25 and 30°C, *S. aureus* growth was more obvious than at 15°C. The μ_{max} values (0.11-0.18 log CFU·g^{-1}·h^{-1}) were similar for mozzarella and cheddar cheese at 25°C, but mozzarella cheese had significantly higher ($P < 0.05$) μ_{max} (0.52 log CFU·g^{-1}·h^{-1}) than cheddar cheese (0.16 log CFU·g^{-1}·h^{-1}) at 30°C (Table 1). To investigate the difference in kinetic behavior between the two cheeses, pH and a_w were measured. However, no difference

Table 1. Growth parameters (mean ± standard error) of *Staphylococcus aureus* on mozzarella and cheddar cheese, calculated by the Baranyi model[20]

Cheese	Storage temperature (°C)	LPD (h)	μ_{max} (Log CFU·g^{-1}·h^{-1})	N_0 (Log CFU/g)	N_{max} (Log CFU/g)	R^2
Mozzarella	15	48.00 ± 1.63^{B}	0.02 ± 0.00^{C}	3.5 ± 0.0^{A}	8.4 ± 0.1^{A}	0.964-0.996
	25	5.69 ± 3.85^{C}	0.18 ± 0.05^{B}	3.0 ± 0.2^{BC}	8.8 ± 0.2^{A}	0.956-0.989
	30	3.10 ± 0.71^{C}	0.52 ± 0.03^{A}	2.9 ± 0.1^{C}	8.0 ± 0.1^{A}	0.961-0.973
Cheddar	15	130.01 ± 16.59^{A}	0.01 ± 0.02^{C}	3.1 ± 0.1^{B}	4.9 ± 0.2^{A}	0.785-0.859
	25	21.98 ± 2.07^{C}	0.11 ± 0.02^{BC}	3.3 ± 0.1^{AB}	7.5 ± 0.1^{A}	0.946-0.958
	30	0.08 ± 0.08^{C}	0.16 ± 0.01^{B}	2.4 ± 0.1^{D}	8.3 ± 0.8^{B}	0.977-0.981

μ_{max}: maximum specific growth rate, LPD: lag phase duration, N_0: lower asymptote, N_{max}: upper asymptote
A-D: different letters in a same column mean significantly different at $P < 0.05$.

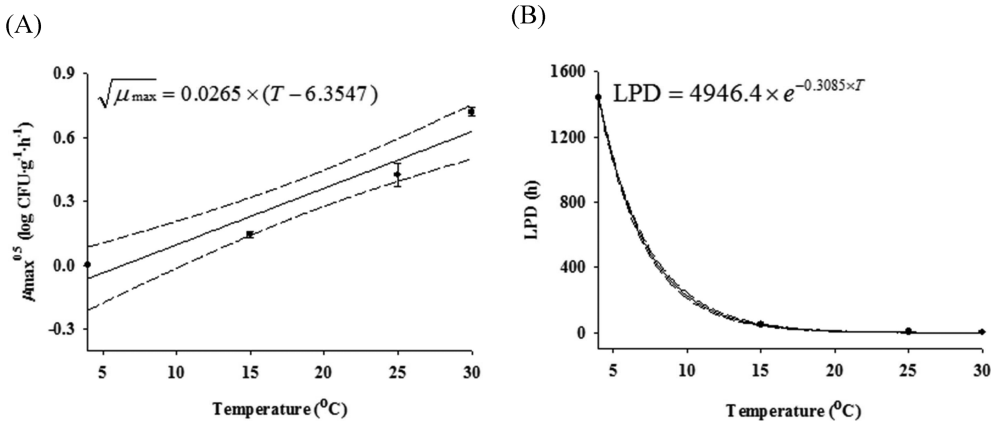

Fig. 1. Secondary model for μ_{max} (A) and LPD (B) developed for mozzarella cheese. •: observed value; -: predicted value; -- : 95% confidence interval.

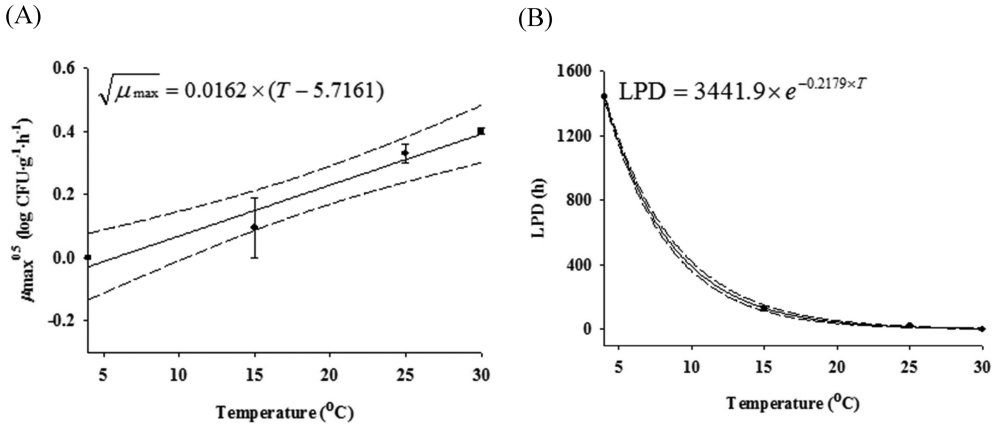

Fig. 2. Secondary model for μ_{max} (A) and LPD (B) developed for cheddar. •: observed value; -: predicted value; -- : 95% confidence interval.

was found in either pH (6.18-6.36) or a_w values (0.965-0.970) between the two cheeses. Therefore, further research is necessary to explain this phenomenon. Since R^2 values ranged from 0.785 to 0.996, the developed primary model was considered an acceptable fit of the data (Table 1).

The effect of storage temperature on kinetic parameters was evaluated by fitting the square root model for kinetic parameters as a function of storage temperature. This showed that the square root of μ_{max} and LPD increased as the storage temperature increased (Figs. 1 and 2). Similarly, a study by Lee *et al.*[22] also showed that the square root of μ_{max} and square root of LPD for *S. aureus* are correlated with storage temperature. The T_{min} values for *S. aureus* were 6.35°C and 5.72°C for the mozzarella and cheddar slice cheeses, respectively (Figs. 1 and 2). In other studies, the T_{min} values for *S. aureus* were 5.82, 5.2, and 0.29°C in milk, carbonara sauce, and octopus sauce, respectively[22]. These results indicate that the theoretical minimum temperature for *S. aureus* varies depending on the bacterial strain and the food it is growing on. Furthermore, Dengremont and Membre[23] found a variation in the T_{min} (5.46-8.70°C) among five strains of *S. aureus* grown in culture medium. The secondary model describing the effect of the storage temperatures on kinetic parameters, provided a good fit for the observed values of μ_{max} and LPD for the mozzarella and cheddar slice cheeses (R^2 = 0.879-0.999) (data not shown).

To evaluate the performance of the developed models, an independent study was conducted at 23°C and 28°C. The *S. aureus* cell counts observed at given storage temperatures (23°C and 28°C) in the independent study, were compared with the cell counts predicted by the developed model. Bias factor and accuracy factors have been generally used, but Oscar[24] recently suggested that those factors have critical limitations. Hence, the RMSE was used to evaluate the model performance in our study. The RMSE values were 0.5344 and 0.3500 for the mozzarella and cheddar slice cheeses, respectively, the lower values of RMSE indicating that the developed model is more appropriate. This result indicates that the developed models are appropriate to predict *S. aureus* cell counts in the mozzarella and cheddar slice cheeses.

In conclusion, the results should be useful in predicting the growth pattern of *S. aureus* on processed cheeses. Moreover, this information could be useful in the microbiological exposure assessment for processed cheeses.

Acknowledgement

This work was supported by Research and Development in Veterinary Science, QIA (Animal, Plant and Fisheries Quarantine and Inspection Agency).

요 약

본 연구는 가공치즈에서 *Staphylococcus aureus*의 생장을 예측하기 위한 수학적 모델을 개발하였다. 모짜렐라 슬라이스 치즈와 체다 슬라이스 치즈에 *S. aureus* 혼합균액(ATCC13565, ATCC14458, ATCC23235, ATCC27664, NCCP10826) 0.1 ml (log CFU/g)을 접종한 후 4°C (1440 h), 15°C (288 h), 25°C (72 h), and 30°C (48 h)에 저장하면서 총 세균 수와 *S. aureus* 세균 수를 tryptic soy agar와 mannitol salt agar를 이용해 각각 확인하였다. *S. aureus*의 세균 수를 Baranyi model로 분석하여 생장률(μ_{max}; log CFU·g^{-1}·h^{-1}), 유도기(LPD; h), 초기 세균수(log CFU/g), 최대 생장 세균수(log CFU/g)를 계산함으로써 1차 모델을 개발하였다. 또한 저장온도와 *S. aureus*의 μ_{max}, LPD의 관계를 분석하기 위해 square root model과 exponential decay model을 이용하였고 이를 통해 2차모델을 개발하였으며 개발된 모델의 평균제곱근 편차(RMSE)를 계산하여 적합성을 검증하였다. 4°C에서는 모든 가공치즈에서 황색포도상구균의 생장이 관찰되지 않았으나 15°C, 25°C, 30°C에서는 모짜렐라 슬라이스와 체다 슬라이스 치즈에서 황색포도상구균이 생장하였으며(R^2 = 0.785-0.996) 저장온도가 높아짐에 따라 생장률은 증가한 반면 유도기는 감소하였다(R^2 = 0.879-0.999). 또한 개발된 모델의 RMSE 값은 0.3500-0.5344로 적합하였다. 따라서 본 연구결과는 가공치즈에서 황색포도상구균의 생장 예측에 유용하게 사용될 것이다.

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