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Research Note

Predictive Model for Growth of *Staphylococcus aureus* on Raw Pork, Ham, and Sausage

AHMAD ROIS MANSUR,† JOONG-HYUN PARK, AND DEOG-HWAN OH*

Department of Food Science and Biotechnology, School of Bioconvergence Science and Technology, Kangwon National University, Chuncheon, Gangwon 200-701, Republic of Korea

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ABSTRACT

Recent *Staphylococcus aureus* outbreaks linked to meat and poultry products underscore the importance of understanding the growth kinetics of *S. aureus* in these products at different temperatures. Raw pork, ham, and sausage (each 10 ± 0.3 g) were inoculated with a three-strain cocktail of *S. aureus,* resulting in an initial level of ca. 3 log CFU/g. Samples were stored isothermally at 10, 15, 20, 25, 30, 35, and 40°C, and *S. aureus* was enumerated at appropriate time intervals. The square root model was developed using experimental data collected from *S. aureus* grown on all samples (where data from raw pork, ham, and sausage were combined) so as to describe the growth rate of *S. aureus* as a function of temperature. The model was then compared with models for *S. aureus* growth on each individual sample in the experiments (raw pork, ham, or sausage) and the *S. aureus* ComBase models, as well as models for the growth of different types of pathogens (*S. aureus, Escherichia coli* O157:H7, *Clostridium perfringens, Salmonella* serovars, and *Salmonella* Typhimurium) on various types of meat and poultry products. The results show that the *S. aureus* model developed here based on the pooled data from all three pork products seems suitable for the prediction of *S. aureus* growth on different pork products under isothermal conditions from 10 to 25°C, as well as for *S. aureus* growth on different meat and poultry products at higher temperatures between 20 and 35°C. Regardless of some high deviations observed at temperatures between 25 and 40°C, the developed model still seems suitable to predict the growth of other pathogens on different types of meat and poultry products over the temperature ranges used here, especially for *E. coli* O157:H7 and *Salmonella* Typhimurium. The developed model, therefore, may be useful for estimating the effects of storage temperature on the behavior of pathogens in different meat and poultry products and for microbial risk assessments evaluating meat safety.

Pork is the most highly consumed meat in the world *(8)*. In the European Union, people eat more pork than any other meat *(15)*. In China, the consumption of pork has also continued to increase with economic development. Reports from 1989 to 1993 show that the daily consumption of pork increased from 0.18 to 0.21 pounds per person *(9)*. According to another report, from August 2013 to August 2014, the United States exported over 5.5 million pounds of pork, making the U.S. swine industry an important form of trade *(21)*. For these reasons, ensuring the safety of the pork supply chain is crucial.

Although pork has been less associated with foodborne illness than other meat sources, it remains of significant concern due to the large consumption *(8)*. *Salmonella* serovars and *Staphylococcus aureus* are among the top pathogens causing foodborne illness and death annually and have been well documented to be present in pigs or pork products. These facts make pork a potential contributor to foodborne illness *(1)*. *S. aureus* contamination itself is typically associated with the handling of meat products after processing. Moreover, the *S. aureus* strains causing foodborne illness are of human origin, not from the raw meat or livestock *(12)*. For instance, in 2005, an outbreak of *S. aureus* in southeast Kansas occurred at a catered event. Smoked sausage was implicated as the source of infection, which was likely the result of contaminated equipment or humans, combined with improper cooling, reheating, or holding of the product *(10)*.

If contaminated meat or poultry or their products are stored at elevated temperatures, pathogens can grow to reach high concentrations within 24 h, without overt visual signs of spoilage. In recent years, there have been *S. aureus* outbreaks linked to meat and poultry dishes in the United States *(3),* and several other foodborne diseases and illness outbreaks related to meat and poultry products. For instance, the consumption of contaminated meat, poultry, and associated products accounted for 12.2 to 18.3% of salmonellosis cases in 2004 and 2005. Meat was also found to be a substantial (11.2 to 25.0%) source of bacterial toxins produced by microorganisms like *Clostridium perfringens (15)*. Furthermore, the pathogen-commodity pairs most commonly responsible for outbreaks were *Salmonella* and poultry (145 outbreaks), *C. perfringens* and poultry (3,452 illnesses), and *C. perfringens* and beef (2,963 illnesses) *(6)*.

* Corresponding author. Tel and Fax: +82 33 250 6457; E-mail: deoghwa@kangwon.ac.kr.
† Present address: Food Analysis Center, Korea Food Research Institute, Anyangpangyo, Bundang, Seongnam, Gyeonggi 463-746, Republic of Korea.

Modeling the growth of various pathogens that can influence or contribute to the risk of multiple types of meats and meat products is therefore necessary.

Various growth models of *S. aureus, Salmonella* serovars, *Escherichia coli* O157:H7, and *C. perfringens* on meat, poultry, and their products have been developed in recent years *(4, 5, 11, 13, 14, 16, 19)*. All of these models have delivered good predictions compared with observations made under isothermal conditions, but they were developed and validated for use in just one type of meat or meat product. In addition, to date, predictive models developed using microbiological growth data generated in one type of meat or meat product are not yet applicable to estimate bacterial growth in different types of meats and meat products. It is therefore important to study the relationship between the *S. aureus* growth model developed in the current study and the published growth models established for other pathogens in various types of meats and meat products so as to investigate the possibility that the *S. aureus* model developed here may be applicable for the prediction of different pathogens grown in various types of meats and meat products.

The purpose of this study, therefore, was to develop a mathematical model capable of predicting the growth of *S. aureus* on raw pork, ham, and sausage as a function of temperature and to associate the observed growth rates with those generated from ComBase. The model was also compared with existing growth models for *S. aureus, Salmonella, E. coli* O157:H7, and *C. perfringens* on meat, poultry, and their products *(4, 5, 11, 13, 14, 16, 19)*.

MATERIALS AND METHODS

Culture preparation. Three strains of *S. aureus* (ATCC 12598, ATCC 12600, and ATCC 25923) were obtained from the Korean National Institute of Health (Seoul, Republic of Korea). Prior to use, each strain was grown separately in tryptic soy broth (TSB; Difco, BD, Sparks, MD) at 37°C with two consecutive transfers after a 24-h period for a total 48 h of incubation. All working cultures grown in TSB were centrifuged separately at 4,000 × *g* for 10 min at 4°C, and the supernatants were discarded. The cell pellets were washed twice with 0.1% sterile buffered peptone water (Difco), pH 7.1, and resuspended in 10 ml of the same solution to obtain a final cell concentration of ca. 5 log CFU/ml. The three strains were then combined to make a cocktail with approximately equal numbers in the final population (ca. 5 log CFU/ml). The bacterial population in each culture cocktail was confirmed by plating 0.1-ml portions of appropriately diluted culture on tryptic soy agar (Difco) and incubating them at 37°C for 24 h.

Sample preparation. Boneless pork loin, fresh ham, and sausage were purchased from a local supermarket (Lotte Mart, Chuncheon, Korea) and transported to the laboratory under temperature-controlled conditions in a sterile plastic container. Each sample (raw pork, ham, or sausage) was then separately cut into pieces using a sterile knife. Aliquots with a weight of 10 ± 0.3 g were used for the storage tests.

Inoculation and storage. Each sample (10 ± 0.3 g) was spot inoculated by pipetting 0.1 ml of each culture cocktail (ca. 5 log CFU/ml) onto the surface to obtain an initial level of ca. 3 log CFU/g. The inoculated samples were then air dried in the laminar flow hood for 1 h at 23 ± 2°C to allow attachment of the bacteria. After drying, the samples were separately transferred into sterile stomacher bags (Whirl-Pak, Nasco, Janesville, WI) and stored isothermally at one of seven temperatures (10, 15, 20, 25, 30, 35, or 40°C).

Bacterial enumeration. During storage, subsamples were analyzed at 6- to 12-h intervals for the samples stored at 10°C and at 1-h intervals for those stored at 40°C. Generally, samples stored at lower temperatures had longer sampling intervals, while shorter intervals were selected for samples at higher temperatures. At each sampling point, samples were mixed with 90 ml of 0.1% sterile buffered peptone water and homogenized for 2 min in a Seward stomacher (400 Circulator, Seward, London, UK). After homogenization, 1-ml aliquots of each sample were serially diluted in 9 ml of 0.1% sterile buffered peptone water, and 0.1-ml amounts of the diluents were spread plated onto Baird Parker agar (Difco) supplemented with 50 ml of egg yolk Tellurite emulsion. All plates were then incubated at 37°C for 24 h. Each experiment consisted of three independent trials, with duplicate analyses in each trial.

Model development. A model was developed using the experimental data collected from *S. aureus* grown on all samples, combining data from raw pork, ham, and sausage. Bacterial counts from Baird Parker agar medium were used in the model fitting. DMFit Excel Add-in software (Institute of Food Research, Norwich, UK) was used to model the growth from all experimental observations, fitting the data to the Baranyi and Roberts model *(2)*, and the growth of *S. aureus* was expressed as a function of time. Ratkowski's square root model *(17, 18)* was then used to describe the maximum growth rate (μ_{max}, log CFU/h) of *S. aureus* as a function of temperature as follows:

$$\sqrt{\mu_{max}} = c \times (T - T_0)$$

where c is the regression coefficient, T is the temperature (°C), and T_0 is the theoretical minimum temperature for bacterial growth.

Model comparison. The developed growth model here was compared with the growth models for *S. aureus* grown on each individual sample (raw pork, ham, or sausage) and the *S. aureus* ComBase models. The use of the ComBase models requires the water activity (a_w) and pH values of the samples to be measured. The average pH and a_w values of raw pork, ham, and sausage were 5.77 ± 0.12 and 0.97 ± 0.01, respectively.

The growth model developed was also compared with several existing growth models for pathogens on various types of meat, poultry, and their products, as shown in greater detail in Table 1. The square root growth rate (log CFU per gram per hour) data from all the models were used for comparison so as to investigate the relationship between them *(7)*. Comparisons were only made in the range of temperatures examined here (from 10 to 40°C).

RESULTS AND DISCUSSION

The growth curves of *S. aureus* on raw pork, ham, and sausage stored under various isothermal conditions (10, 15, 20, 25, 30, 35, and 40°C) are shown in Figure 1. The growth curves were produced using the primary growth model of Baranyi and Roberts *(2)*, which showed a high correlation coefficient ($R^2 > 0.94$), except for those obtained from ham and sausage stored at 10°C ($R^2 > 0.64$ and $R^2 > 0.47$, respectively). The μ_{max} of *S. aureus* on each pork sample

TABLE 1. *Pathogen growth models used for comparison with the* Staphylococcus aureus *growth model developed here*

Model	Pathogen	Type of food sample	Primary model	Reference or source
1	*Staphylococcus aureus*	Raw pork	Baranyi and Roberts model	This study
		Ham	Baranyi and Roberts model	This study
		Sausage	Baranyi and Roberts model	This study
		Raw pork	Baranyi and Roberts model	*11*
		RTE pork	Modified Gompertz model	*16*
		Bratwurst	Baranyi and Roberts model	*4*
2	*Staphylococcus aureus*	Ground beef	Baranyi and Roberts model	*4*
		Ground turkey	Baranyi and Roberts model	*4*
		Cooked turkey breast	Baranyi and Roberts model	*5*
		Cooked chicken breast	Baranyi and Roberts model	*5*
3	*Salmonella* Typhimurium	RTE pork	Modified Gompertz model	*16*
	Salmonella serovars	Bratwurst	Baranyi and Roberts model	*4*
		Raw pork	Baranyi and Roberts model	*11*
	Escherichia coli O157:H7	Bratwurst	Baranyi and Roberts model	*4*
		Raw pork	Baranyi and Roberts model	*13*
4	*Salmonella* serovars	Ground beef	Baranyi and Roberts model	*4*
		Ground turkey	Baranyi and Roberts model	*4*
	Escherichia coli O157:H7	Ground beef	Baranyi and Roberts model	*4*
		Ground beef	Baranyi and Roberts model	*19*
	Clostridium perfringens	Cooked cured chicken	Baranyi and Roberts model	*13*
		Cooked uncured beef	Baranyi and Roberts model	*14*

and at each storage temperature were calculated. The results show that the μ_{max} increased as the storage temperature increased. No naturally occurring bacterial colonies were evident when uninoculated samples were plated on selective medium (data not shown). The square root of μ_{max} (with combined raw pork, ham, and sausage data) was fitted to the Ratkowsky equation [μ_{max} (log CFU/h) = –0.150 × (T – 0.173)] with satisfactory results ($R^2 > 0.986$).

Figure 2 shows a comparison of the *S. aureus* models developed from each individual pork product (raw pork, ham, or sausage) examined here, the models of *S. aureus* growth on bratwurst, raw pork, and ready-to-eat (RTE) pork

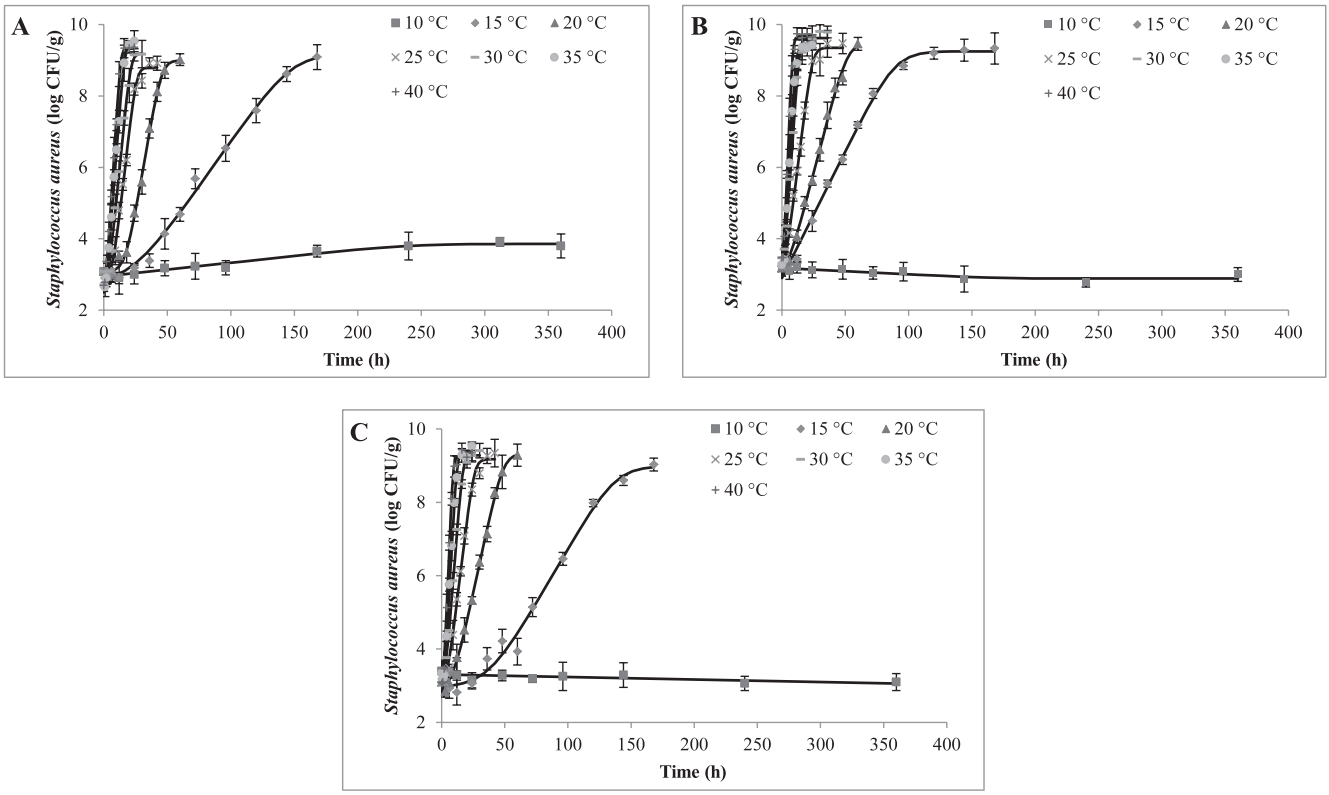

FIGURE 1. *Growth curves of* S. aureus *on raw pork (A), ham (B), and sausage (C) stored under various isothermal conditions (10 to 40°C). Data are the mean results ± standard deviations.*

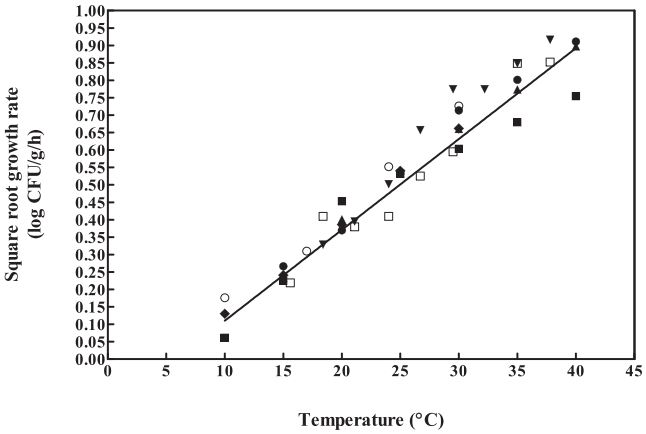

FIGURE 2. *Comparison of* S. aureus *growth rates measured for the model developed here based on data pooled from all three pork products (—), individual raw pork (■), ham (●), and sausage (▲) samples, ComBase growth rate predictions (♦), and the growth rate predictions in the literature for raw pork (□), RTE pork (○), and bratwurst (▼).*

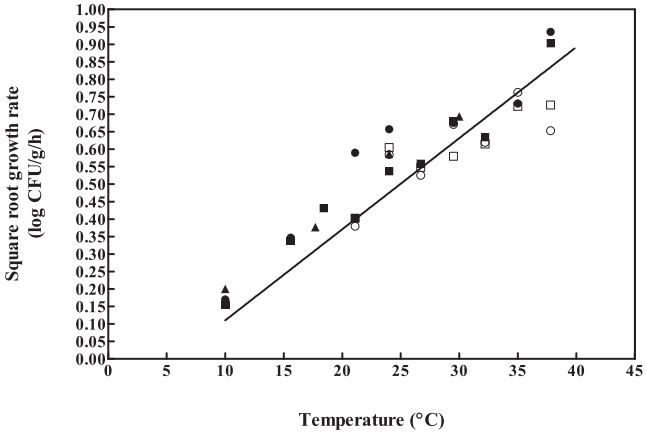

FIGURE 4. S. aureus *growth rates measured for the model based on pooled data from all three pork products (—) compared with the growth rate predictions in the literature for* Salmonella *Typhimurium on RTE pork (▲),* E. coli *O157:H7 on raw pork (■) and bratwurst (□), and* Salmonella *serovars on raw pork (●) and bratwurst (○).*

established by Borneman et al. *(4),* Ingham et al. *(11),* and Min and Yoon *(16),* respectively, and the *S. aureus* growth model developed here using a combination of the data from the three pork products. The *y* axis shows the square root of the growth rate (SQRT) in log CFU per gram per hour, while the *x* axis shows the temperature in degrees centigrade. The SQRT in all models was linear with temperature. Wide spread of the SQRT data occurred at the higher temperatures between 25 and 40°C. Similar growth rates of *S. aureus* were observed for all the models from 10 to 25°C. Despite the putative differences between the different pork products, the *S. aureus* growth rates were similar on all pork products, except on raw pork examined here and bratwurst in the study by Borneman et al. *(4).* Interestingly, the *S. aureus* growth rates obtained by the model based on the pooled data from all three pork products here were quite similar to those predicted by the ComBase, where they were developed using the average pH and water activity values of raw pork, ham, and sausage. The results show that the *S. aureus* growth model developed here using the data from all three pork products seems to have the capability of predicting *S. aureus* growth on different pork products stored at isothermal temperatures from 10 to 25°C. Besides that, we have also successfully validated the ComBase model for its suitability for the prediction of *S. aureus* in pork and pork products.

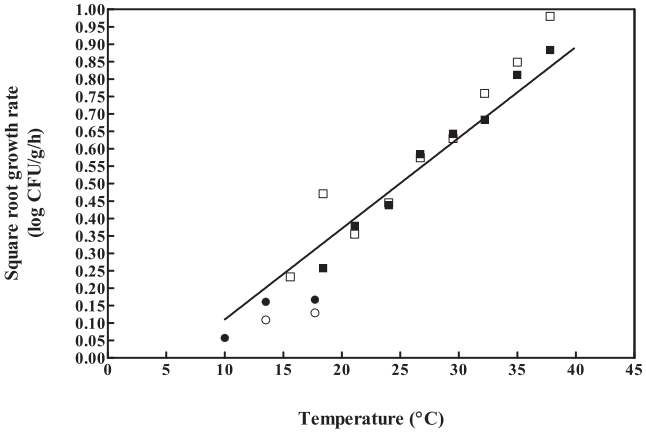

FIGURE 3. S. aureus *growth rates measured for the model based on data pooled from all three pork products (—) compared with the growth rate predictions in the literature for ground beef (■), ground turkey (□), turkey breast (●), and chicken breast (○).*

A comparison of the *S. aureus* models developed for different meat and poultry products is shown in Figure 3. The SQRT data points of the comparison models tend to be equally distributed no matter the temperature. At lower temperatures between 10 and 20°C, the SQRT data obtained from chicken and turkey breast samples *(5)* were much lower than those of the model developed here, whereas at higher temperatures between 20 and 35°C, the SQRT data obtained from ground turkey and beef *(4)* were similar to those of the model developed here. As the temperature rose (between 35 to 40°C), however, the spread between predictions increased, particularly for the SQRT data obtained from ground turkey, which had a greater deviation than the SQRT from ground beef. The results reveal that the *S. aureus* model based on the pooled data from all three pork products also seems capable of predicting the growth of *S. aureus* in different meat and poultry products, while the predictions are likely to be more accurate if the products are stored at higher temperatures between 20 and 35°C.

The *S. aureus* growth model developed here was also compared with other existing pathogen growth models, including *Salmonella* Typhimurium on RTE pork *(16)* and *E. coli* O157:H7 and *Salmonella* serovars on bratwurst *(4)* and raw pork *(11),* as shown in Figure 4. The results show that the *S. aureus* model developed here was nearly identical with the model of *E. coli* O157:H7 and *Salmonella* serovars grown on bratwurst *(4).* The SQRT data of the three models were similar, except for those at high temperatures close to 40°C, in which the SQRT data of the other models were much lower than those of our model. On the other hand,

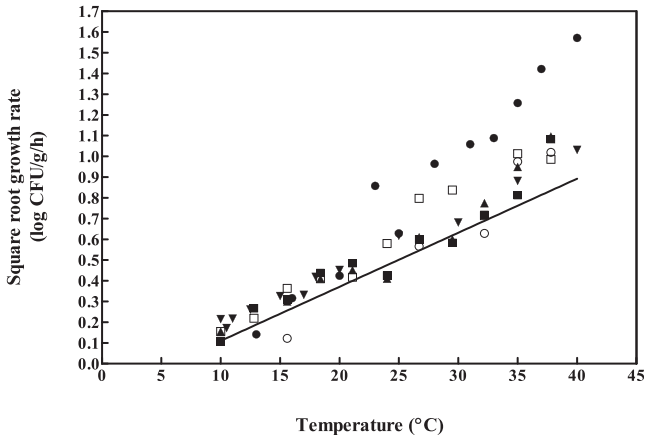

FIGURE 5. S. aureus *growth rates measured for the model based on pooled data from all three pork products (—) compared with the growth rate predictions in the literature for* Salmonella *serovars on ground beef (■) and turkey (□),* E. coli *O157:H7 on beef in two models (▲ and ▼), and* C. perfringens *on cooked, uncured beef (●) and cured chicken (○).*

wide spreads between the SQRT data of *E. coli* O157:H7 and *Salmonella* serovars on raw pork compared with those of the *S. aureus* model developed here were observed. In addition, the *Salmonella* Typhimurium growth model on RTE pork established by Min and Yoon *(16)* shows slightly greater SQRT predictions than the *S. aureus* growth model. Overall, most of the models were fairly similar, given that different strains, laboratories, and methods were used in their creation. Thus, the *S. aureus* growth model developed here is likely suitable for predicting the growth of other pathogens (especially *E. coli* O157:H7 and *Salmonella* Typhimurium) in pork and pork products over the temperature range of interest examined here.

Figure 5 shows a comparison of the data for growth models of various pathogens in different meat and poultry products. The *S. aureus* growth model developed here was compared with the models for *Salmonella* serovars in ground beef and turkey *(4), E. coli* O157:H7 in ground beef *(4)* and beef *(19),* and *C. perfringens* in cooked, uncured beef *(13)* and cured chicken *(14).* The spread between the SQRT predictions increased with rising temperature, especially at the temperatures between 25 and 40°C. *C. perfringens* growth in cooked, cured beef had the widest spread, followed by the models of *C. perfringens* in cooked, cured chicken and of *Salmonella* serovars in ground turkey. In contrast, the SQRT predictions of the *Salmonella* serovars and *E. coli* O157:H7 models in ground beef were fairly similar to those of the *S. aureus* model developed here, especially at the temperatures between 10 and 35°C. The results also indicated that the *S. aureus* growth model seems to have limitations when used to predict the growth of other pathogens in various meat and poultry products, especially at temperatures between 25 and 40°C. Natural variability and additional factors affecting bacterial growth not included in the model design, such as the presence of contaminating microorganisms, the addition of preservatives, and modified atmosphere packaging, etc., may also account for the lack of similarity between the developed models *(20).*

In conclusion, a mathematical model describing the SQRT of *S. aureus* on all three pork products (where data from raw pork, ham, and sausage were combined) as a function of temperature was developed. The SQRT predictions for *S. aureus* in the developed model were compared with those from the ComBase predictor and other pathogen growth models obtained from several selected sources in the literature. The results show that the *S. aureus* growth model based on the pooled data from all three pork products seems suitable for the prediction of *S. aureus* growth on different pork products under isothermal conditions from 10 to 25°C and of *S. aureus* growth on different meat and poultry products under higher temperatures from 20 to 35°C. Regardless of some high deviations observed at the temperatures between 25 and 40°C, the developed model is still likely suitable for predicting the growth of other pathogens on meat and poultry products over the temperature range of the experiments conducted here, especially for *E. coli* O157:H7 and *Salmonella* Typhimurium. This study, therefore, may provide a fast and cost-effective alternative to laboratory studies to estimate the effects of storage temperature on pathogen behavior in different meat and poultry products and may also be used in subsequent quantitative microbial risk assessments evaluating meat safety.

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