# Development of the Model to Predict the Growth of *Salmonella amsterdam* and *Salmonella bangkok* in Stirred Fried Rice with Crab Meat

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## ABSTRACT

***Central Composite Design (CCD) was used to study the main factors (temperature, pH, salt concentration, initial innoculum and incubation time) affecting Salmonella enteritidis growth in stirred fried rice with crab meat (Khao Pad Pu). The result obtained from the CCD fitting by a second-order model, using a quadratic polynomial equation, was as follow:***

$Y = 37.024 – 0.005\ INOC + 7.7 \times 10^{-7}\ INOC^2 + 0.001\ INOC.\ pH + 3.5 \times 10^{-4}\ INOC.Time + 3.131\ NaCl + 7.713\ NaCl^2 - 6.8 \times 10^{-5}\ NaCl.INOC – 2.20\ NaCl.pH + 0.148\ NaCl.\ Time - 11.0\ pH + 0.0927\ pH^2 + 0.037\ pH.Time + 0.489\ Temp - 0.003\ Temp^2 - 2.3 \times 10^{-4}\ Temp.\ INOC - 0.056\ Temp.NaCl - 0.019\ Temp.pH + 0.007\ Time.Temp - 0.619\ Time - 6.00 \times 10^{-4}\ Time^2.$

$R\text{-}square = 0.8659$

***This equation was used to predict the population of Salmonella amsterdam and Salmonella bangkok in Khao Pad Pu, growing under different conditions. The predicted values were compared with viable counts for validation. It was found that the $R^2$ were 0.618 and 0.691 for S. amsterdam and S. bangkok, respectively.***

**Key words:** *Salmonella*, Growth model, Central Composite Design (CCD)

## INTRODUCTION

All *Salmonella* strains are potentially pathogenic to human. *Salmonella* has O, H and V antigens. *Salmonella* grows at the temperature of 5°-47°C, pH 3.6–9.5 and $a_w$ between 0.93 –0.99. The heat at 62°C for 4 minutes can kill *Salmonella* in food (Labbe and Garcia, 2001). *Salmonella* is one of the main causes of diarrheal disease in Thailand. *Salmonella* species in 6 serovars were detected in yum ruam mit, khao pad pu and yum pla duk phoo from 3 supermarkets in Bangkok (Boonyaratanakornkit et al., 2000). Several mathematical models have been applied to predict the effects of temperature, pH, water activity and other factors on bacterial

growth rate (Meng and Schaffner ,1997; Nyati, 2000; Gardini et al., 2001). Latimer et al., (2002) studied growth model of *Salmonella enteritidis* (SE) by combining two mathematical equations that described both the extended lag phase of SE growth (food component) and SE growth model (pathogen component). In the final step of model development, the best-fitting secondary models for growth of microorganism were combined in a computer spreadsheet to create a tertiary model that predicted the potential growth of microorganism as a function of variables.

This research work applied mathematical model used for S. enteritidis to test *S. amsterdam* and *S. bangkok* growth under various conditions in Khao Pad Pu.

## MATERIALS AND METHODS

### Experimental Design

According to the Central Composite Design (CCD), S. amsterdam and S. bangkok were grown under 160 different conditions of five variables (pH, temperature, incubation time, number of inoculation and NaCl concentration).

### Preparation of Khao Pad Pu

Khao Pad Pu was prepared as follow: All ingredients were mixed and stirred while frying. NaCl was added to provide final concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent (w/w). Khao Pad Pu was divided into portions (25 g) in the plastic bags and sterilized. The amount of sterile 0.2 per cent citric acid was added to obtain pH of 5.2, 5.7, 6.2, 6.7 and 7.2. Then, the samples were thoroughly mixed and inoculated with *Salmonella* to obtain the inoculum size of 100, 325, 550, 775, and 1,000 cfu /g. Then, they were incubated at the temperature of 5, 15, 25, 25 and 45°C. Finally, they were sampled at 1h, 6h 45min, 12h 30min, 18h 15min and 24 h to determine number of *Salmonella* by using AOAC (1995) official method.

### Model Equation

Logarithmic numbers of *Salmonella* were calculated out by using statistic SWX 7.0 software. The model fitted for *S. enteritidis* was used to predict growth of *S. amsterdam* and *S. bangkok*. The polynomial equation was as follow:

$Y = 37.024 – 0.005\ INOC + 7.7 \times 10\text{-}7\ INOC^2 + 0.001\ INOC.pH + 3.5\times10^{-4}\ INOC.Time + 3.131\ NaCl + 7.713\ NaCl^2 - 6.8\times10^{-5}\ NaCl.INOC – 2.20\ NaCl.pH + 0.148\ NaCl.Time - 11.0\ pH + 0.0927\ pH^2 + 0.037\ pH.Time + 0.489\ Temp - 0.003\ Temp^2 - 2.3\times10^{-4}\ Temp.INOC - 0.056\ Temp.NaCl - 0.019\ Temp.pH + 0.007\ Time.Temp - 0.619\ Time - 6.00\times10^{-4}\ Time^2.$

R-square = 0.8659

where Y is logarithmic numbers of *Salmonella* (cell/g), INOC is inoculum size (cell/g), Temp is temperature (°C), NaCl is NaCl (% w/w) and Time is incubation time (hour). The equation has 1 constant, 5 independent variables and 19 coefficients. The model was a linear regression (Pongsomboon et al., 2004).

### Validation of Model

*S. amsterdam* and *S. bangkok* were grown under different conditions. To validate the model, the predicted data were calculated out by using the second-order model of polynomial equation as shown above and compared to the observed data from the experiments.

## RESULTS

The comparison between observed and predicted data of *S. amsterdam* is shown in Fig. 1. The $R^2$ was 0.618. The model accounted for 61.8 per cent of the variability in the experiment.

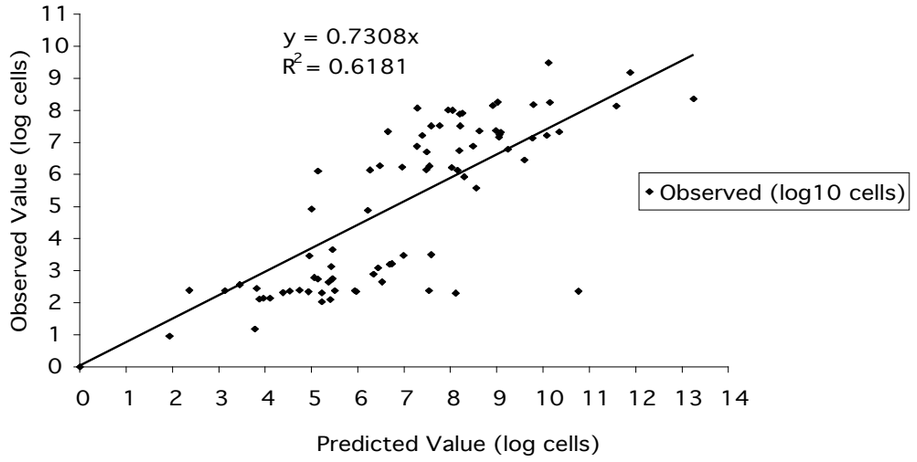


**Figure 1.** The Predicted Value ($log_{10}$ cells) vs. Observed Value ($log_{10}$ cells) of *S. amsterdam* in Khao Pad Pu.

The comparison between observed and predicted data of *S. bangkok* is shown in Fig. 2. The $R^2$ was 0.691. The model accounted for 69.1 per cent of the variability in the experiment.

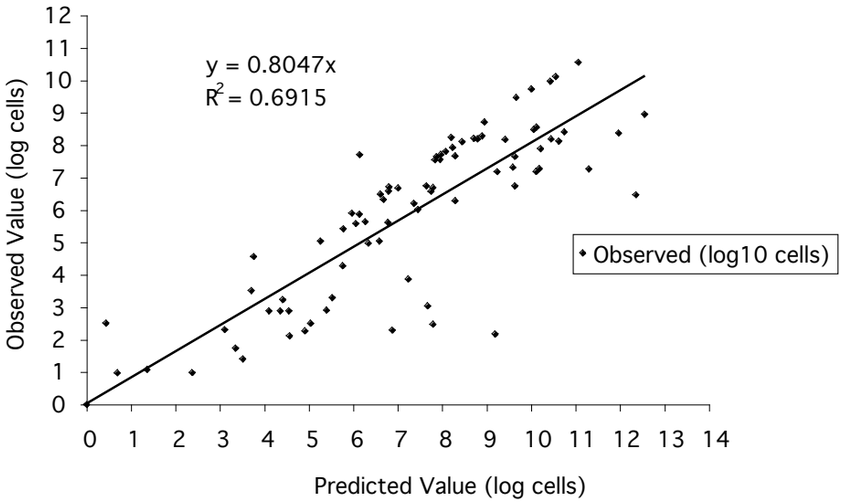


**Figure 2.** The Predicted Value ($log_{10}$ cells) vs. Observed Value ($log_{10}$ cells) of S. bangkok in Khao Pad Pu.

## DISCUSSION AND CONCLUSION

The comparison of the predicted to the observed growth of *S. amsterdam* and *S. bangkok* in Khao Pad Pu was accounted for 61.8 and 69.1 per cent, respectively. Skandamis and Nychas (2000) reported the percentage between observed and predicted data of *Escherichia coli* 0157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pH and oregano essential oil concentrations as 76.7 per cent. Meng and Schaffner (1997) showed that quadratic polynomial models for germination, outgrowth and lag time (GOL) and exponential growth rate (EGR) of *Bacillus stearothermophilus* in terms of temperature, pH and NaCl were generated by response surface analysis. The $R^2$ values for GOL and EGR model were 0.917 and 0.916, respectively. Basti and Razavilar (2004) predicted some growth kinetics of *Salmonella typhimurium*, time-to-detection (TTD) and log probability percentage (log P%) of growth initiation models which were used to measure the effects of pH (5.0–7.4), sodium chloride (NaCl, 0.5–3%), potassium sorbate (KS, 0.0–0.3%) and temperature (T, 15–35°C ) on the growth response of this organism. They found that between two models obtained in the study (TTD and log P%), the TTD model showed better prediction ($R^2$ =0.91) than the logP% model of growth ($R^2$ = 0.73).

In the present study, the comparison of predicted and observed values of *S. amsterdam* and *S. bangkok* showed that at high temperature (45°C), the observed values were lower than the predicted values. It was obvious that the model did not respond well. The reason for this is that the model is developed for the growth of *S. enteritidis* which is the different species from *S. amsterdam* and *S. bangkok* as shown in Table 1. In addition, the temperature at 45°C may be too high for *S. amsterdam* and *S. bangkok* to grow. So, this mathematical model should be further developed to fit other *Salmonella* species.

**Table 1.** Different Characters of *Salmonella* species.

| Salmonella | Group | Somatic (O) antigen | Flagellar (H) antigen |
|---|---|---|---|
| *S. enteritidis* | O ; 9 ($D_1$) | 1, 9, 12 | g, m |
| *S. amsterdam* | O ; 3, 10 ($E_1$) | 3, 10[15] [15,34] | g, m, s |
| *S. bangkok* | O ; 38 (P) | 38 | $Z_4$ , $Z_{23}$ |

Source : Popoff and Le Minor (1997).

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