ORIGINAL ARTICLE

Development of the model to predict the growth of *Salmonella* spp. in stirred fried rice with crab meat

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Abstract

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Central Composite Design (CCD) was used to study the main factors (temperature, pH, salt concentrations, initial inoculums and incubation time) affecting Salmonella enteritidis growth in stirred fried rice with crab meat. The result obtained from the CCD, fits in the second-order model using a quadratic polynomial equation.

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

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The model is developed for wider application. The new equation is as follows:

$$Y = 37.024 - 0.005\ INOC + 0.001\ INOC.\ pH + 3.5x10^{-4}\ INOC.Time + 3.131\ NaCl + 7.713\ NaCl^2 - 2.20\ NaCl.pH + 0.148\ NaCl.Time - 11.0\ pH + 0.0927pH^2 + 0.037\ pH.Time + 0.489\ Temp - 0.003\ Temp^2 - 2.3x10^{-4}\ Temp.INOC - 0.056\ Temp.NaCl - 0.019\ Temp.pH + 0.007\ Time.Temp - 0.619\ Time\ .$$

This new model is used to predict the population of *Salmonella enteritidis, Salmonella amsterdam, Salmonella bangkok* and *Salmonella dublin* in stirred fried rice with crab meat grew at different conditions. It is found that the new model accuracy is 94.93, 79.36, 88.39 and 96.33 per cent to predict growths of of *Salmonella enteritidis, Salmonella amsterdam, Salmonella bangkok* and *Salmonella Dublin,* respectively.

Key words : *Salmonella*, growth model, central composite design

บทคัดย่อ

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การพัฒนาแบบจำลองเพื่อใช้ทำนายการเจริญของ *Salmonella* spp. ในข้าวผัดปู

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แผนการทดลองแบบ Central Composite Design (CCD) จึงถูกนำมาศึกษาปัจจัยหลัก (อุณหภูมิ pH ความเข้มข้นเกลือ เชื่อที่เริ่มต้น และเวลา) ที่เกี่ยวกับการเจริญของ *Salmonella enteritidis* ในข้าวผัดปู พบว่า ในการวางแผนการทดลองแบบ CCD จะให้ผลสอดคล้องกับแบบจำลองแบบกำลังสอง ดังสมการ

$$Y = 37.024 - 0.005\ INOC + 7.7 \times 10^{-7}\ INOC^2 + 0.001\ INOC.\ pH + 3.5x10^{-4}\ INOC.Time + 3.131\ NaCl + 7.713\ NaCl^2 - 6.8x10^{-5}\ NaCl.INOC - 2.20\ NaCl.pH + 0.148\ NaCl.Time - 11.0\ pH + 0.0927\ pH^2 + 0.037\ pH.Time + 0.489\ Temp - 0.003\ Temp^2 - 2.3x10^{-4}\ Temp.INOC - 0.056\ Temp.NaCl - 0.019\ Temp.pH + 0.007\ Time.Temp - 0.619\ Time - 6.00x10^{-4}\ Time^2.$$

จึงมีการพัฒนาแบบจำลองเพื่อใช้ประโยชน์ให้กว้างขวางขึ้น ดังสมการ

$$Y = 37.024 - 0.005\ INOC + 0.001\ INOC.\ pH + 3.5x10^{-4}\ INOC.Time + 3.131\ NaCl + 7.713\ NaCl^2 - 2.20\ NaCl.pH + 0.148\ NaCl.Time - 11.0\ pH + 0.0927pH^2 + 0.037\ pH.Time + 0.489\ Temp - 0.003\ Temp^2 - 2.3x10^{-4}\ Temp.INOC - 0.056\ Temp.NaCl - 0.019\ Temp.pH + 0.007\ Time.Temp - 0.619\ Time.$$

เมื่อนำแบบจำลองใหม่ทำนายการเจริญของ *Salmonella enteritidis, Salmonella amsterdam, Salmonella bangkok* and *Salmonella Dublin* ในข้าวผัดปูที่สภาวะการทดลองต่าง ๆ พบว่า แบบจำลองใหม่ให้ค่าความแม่นยำเป็น 94.93, 79.36, 88.39 และ 96.33% ตามลำดับ

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Stirred fried rice with crab meat is one of ready-to eat foods. It is sold in supermarkets in the form of foam packaging sealed with film and kept at 8°-10°C. *Salmonella* spp. is one of the main causes of diarrheal 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). *Salmonella entrotica* is further sub-

divided into six subspecies which serovars commonly isolated from humans, agricultural products, and food generally belong to the subspecies enterica (Labbe&Garcia,2001). *Salmonella* has O, H and V antigents. *Salmonella* grows at the temperature 5°C - 47°C, pH 3.6-9.5, and a_w between 0.93-0.99. The heat at 62°C for 4 minutes scan kill *Salmonella* in food. Several mathematical models have been applied to predict the effects of temperature, pH, water activity and other factors on bacterial growth rate (Nyati, 2000; Meng & Schaffner, 1997; 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 a SE growth model (pathogen component). The Central Composite Design (CCD) was used to model the viable count of *Salmonella enterica* in home-made mayonnaise (Xiong *et al.*, 2002). In the final step of model development, the best fitting secondary model for growth of microorganisms was combined in a computer spreadsheet to create a tertiary model that predicted the potential growth of microorganisms as a function of the variables.

This research is carried out for the model to predictive food microbiology. The mathematical models for the effects of temperature, pH, salt concentrations, initial innoculums and incubation time on *Salmonella entritidis, Salmonella amsterdam, Salmonella bangkok* and *Salmonella dublin* growth in stirred fried rice with crab meat are developed.

Materials and Methods

Experimental design

According to the Central Composite Design (CCD) the *Salmonella enteritidis* was grown at different conditions of the five variables at α = 2.378 (pH, temperature, incubation time, number of inoculation and NaCl concentration). The CCD is listed in Table 1.

Preparation of stirred fried rice with crab meat

Stirred fried rice with crab meat was prepared with cooked rice 324 g, crab meat 55 g, egg 99 g, onion leaf 4 g, garlic 7 g, white pepper 3 g, sugar 11 g, soy bean sauce 33 g, and soy bean oil 48 g. All the ingredients were mixed and stirred fried. NaCl was added to give final concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent (w/w). Stirred fried rice with crab meat was divided into portion (25 g) in a plastic bag and sterilized at 121°C for 15 min. The amounts of sterile 0.2 per cent (w/v) citric acid were added to obtain pH of 5.2, 5.7, 6.2, 6.7 and 7.2.

Preparation of the inoculated samples

The *Salmonella enteritidis* or *Salmonella amsterdam* or *Salmonella bangkok* or *Salmonella dublin* was cultivated in peptone water at 37°C for 24 hr. The inoculum sizes of *Salmonella enteritidis* or *Salmonella Amsterdam* or *Salmonella bangkok* or *Salmonella dublin* were calculated by using standard curves for each strain (OD vs cell numbers) and converted to cfu/g. The stirred fried rice with crab meat which adjusted NaCl concentration and pH were thoroughly mixed, inoculated with *Salmonella enteritidis* or *Salmonella Amsterdam* or *Salmonella bangkok* or *Salmonella dublin* to gain the initial inoculum sizes of 100, 325, 550, 775, and 1,000 cfu/g, respectively . Then, they were incubated at the temperatures of 5, 15, 25, 35 and 45°C, respectively. Finally, they were sampled at 1h, 6h 45min, 12h 30min, 18h 15min, and 24 h to determine the viable count of *Salmonella enteritidis* or *Salmonella Amsterdam* or *Salmonella bangkok* or *Salmonella dublin* by using AOAC standard method.

Model equation

Logarithmic numbers of *Salmonella* were calculated by using statistic SWX 7.0 software. A second-order model of the independent variable using a quadratic polynomial equation fits in the results in each run of the CCD at the different dependent variables considered:

$$Y = B_0 + \sum B_i X_i + \sum B_{ii} X_i^2 + \sum B_{ij} X_i X_j$$

Where Y is the dependent variable to be modeled.

Table 1. Five Parameters in CCD of *Salmonella enteritidis*

Run	Temp (°C)	NaCl (%w/w)	Inoc (cell/g)	pH	Time (hour)
1	15	0.4	325	5.7	18.15
2	15	0.4	325	6.7	6.45
3	15	0.4	775	5.7	6.45
4	15	0.4	775	6.7	18.15
5	15	0.8	325	5.7	6.45
6	15	0.8	325	6.7	18.15
7	15	0.8	775	5.7	18.15
8	15	0.8	775	6.7	6.45
9	35	0.4	325	5.7	6.45
10	35	0.4	325	6.7	18.15
11	35	0.4	775	5.7	18.15
12	35	0.4	775	6.7	6.45
13	35	0.8	325	5.7	18.15
14	35	0.8	325	6.7	6.45
15	35	0.8	775	5.7	6.45
16	35	0.8	775	6.7	18.15
17	5	0.6	550	6.2	12.30
18	45	0.6	550	6.2	12.30
19	25	0.2	550	6.2	12.30
20	25	1.0	550	6.2	12.30
21	25	0.6	100	6.2	12.30
22	25	0.6	1000	6.2	12.30
23	25	0.6	550	5.2	12.30
24	25	0.6	550	7.2	12.30
25	25	0.6	550	6.2	1.00
26	25	0.6	550	6.2	24.00
27	25	0.6	550	6.2	12.30
28	25	0.6	550	6.2	12.30
29	25	0.6	550	6.2	12.30
30	25	0.6	550	6.2	12.30

B_0 is the constant. B_i, B_{ii} and B_{ij} are coefficients of the model. X_i and X_j are the independent variables.

Model validation

The *Salmonella enteritidis* was grown in different conditions. To validate the model, the predicted data were calculated out by using the second-order model of polynomial equation and compared to the observed data from the experiments.

Model development

The model was developed. The *Salmonella amsterdam, Salmonella bangkok* and *Salmonella dublin* were grown in different conditions. To validate the model, the predicted data was calculated using the modified model and compared to the observed data from the experiments.

Results and Discussion

Model equation

Logarithmic numbers of *Salmonella enteritidis* are calculated out by using statistic SWX 7.0 software. The quadratic polynomial equation is as

follows :

$$Y = 37.024 - 0.005\ INOC + 7.7 \times 10^{-7}\ INOC^2 + 0.001\ INOC.\ pH + 3.5\times10^{-4}\ INOC.Time + 3.131\ NaCl + 7.713\ NaCl^2 - 6.8\times10^{-5}\ NaCl.INOC - 2.20\ NaCl.pH + 0.148\ NaCl.Time - 11.0\ pH + 0.0927\ pH^2 + 0.037\ pH.Time + 0.489\ Temp - 0.003\ Temp^2 - 2.3\times10^{-4}\ Temp.INOC - 0.056\ Temp.NaCl - 0.019\ Temp.pH + 0.007\ Time.Temp - 0.619\ Time - 6.00\times10^{-4}\ Time^2. \quad (1)$$

Where Y is logarithmic numbers of *Salmonella* (cell/g), INOC is inoculum size (cell/g), Temp is temperature (^{o}C), NaCl is NaCl concentration (% w/w) and Time is incubation time (hour). The equation has 1 constant, 5 independent variables and 19 coefficients. The model is a linear regression.

Model validation

To validate the model, the predicted data was calculated using the second-order model of quadratic polynomial equation and compared with the observed data from the experiments. The result of validation is shown in Figure 1. The R^2 was 0.92. The model accounts for 92 per cent accuracy. Whereas Skandamis & Nychas (2000) reported the percentage between observed data and the predicted of *Escherichia coli* 0157:H7 NCTC 12900 in homemade eggplant salad at various temperature, pH, and oregano essential oil con-centrations was 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 & Razavilar (2004) predicted growth of *Salmonella typhimurium*. It was found that the time-to-detection model showed better prediction (R^2 = 0.91) than the log probability percentage model of growth (R^2 = 0.73).

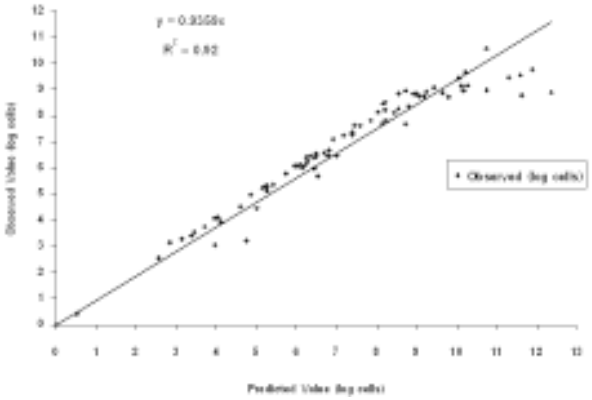

Figure 1. The Predivted Value (log_{10} cells) vs. Observed Value (log_{10} cells) of *Salmonella enteritidis* in Stirred Fried Rice with Crab Meat

Model development

The model is modified by cutting off three coefficients ($7.7 \times 10^{-7}\ INOC^2$, 6.8×10^{-5} NaCl. INOC, $6.00 \times 10^{-4}\ Time^2$) from the original equation (1) due to their low significance in regression analysis. The new equation is as follows:

$$Y = 37.024 - 0.005\ INOC + 0.001\ INOC.\ pH + 3.5\times10^{-4}\ INOC.Time + 3.131\ NaCl + 7.713\ NaCl^2 - 2.20\ NaCl.pH + 0.148\ NaCl.Time - 11.0\ pH + 0.0927\ pH^2 + 0.037\ pH.Time + 0.489\ Temp - 0.003\ Temp^2 - 2.3\times10^{-4}\ Temp.INOC - 0.056\ Temp.NaCl - 0.019\ Temp.pH + 0.007\ Time.Temp - 0.619\ Time. \quad s(2)$$

The new model (2) is used to predict the growth of *Salmonella enteritidis*, *Salmonella amsterdam*, *Salmonella bangkok* and *Salmonella dublin* and compare to the observed data. The results are shown in Figures 2, 3, 4 and 5, respectively. The R^2 for growth of *Salmonella enteritidis*, *Salmonella amsterdam*, *Salmonella bangkok* and *Salmonella dublin* were 0.9493, 0.7936, 0.8839 and 0.9653, respectively. The model accounts for 94.93, 79.36, 88.39 and 96.33 per cent of the accuracy for *Salmonella enteritidis*, *Salmonella amsterdam*, *Salmonella bangkok* and *Salmonella dublin*, respectively. It was reported elsewhere that the range of rapid growth of *Salmonella* was

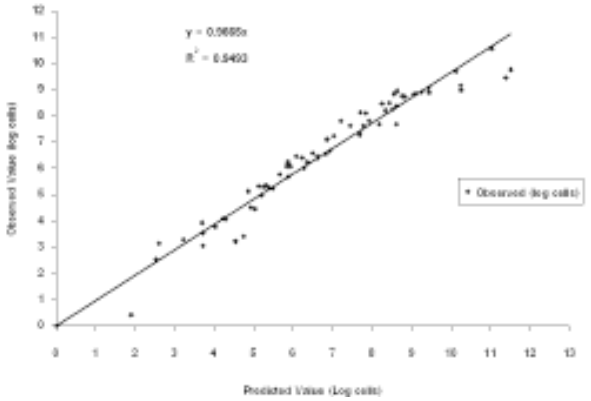

Figure 2. The Predicted Value (log_{10} cells) vs. Observed Value (log_{10} cells) of *Salmonella enteritidis* in Stirred Fried Rice with Crab Meat by New Model (2)

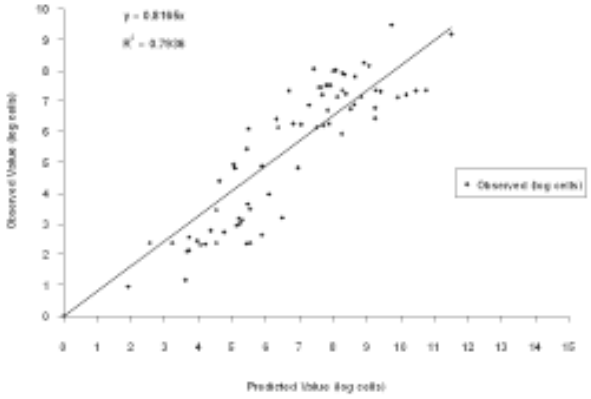

Figure 3. The Predicted Value (log_{10} cells) vs. Observed Value (log_{10} cells) of *Salmonella amsterdam* in Stirred Fried Rice with Crab Meat by New Model (2)

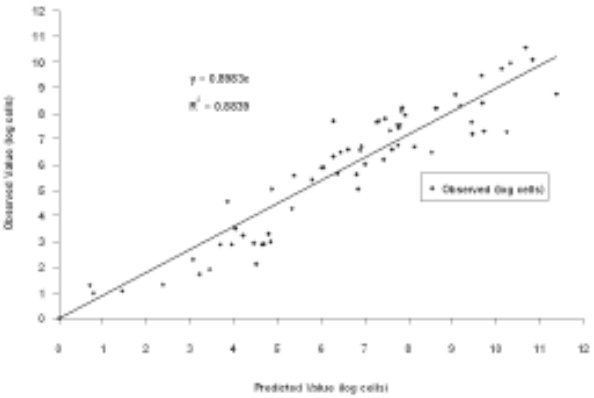

Figure 4. The Predicted Value (log_{10} cells) vs. Observed Value (log_{10} cells) of *Salmonella bangkok* in Stirred Fried Rice with Crab Meat by New Model (2)

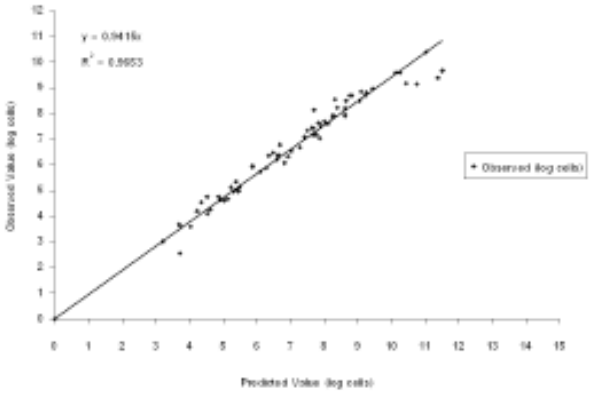

Figure 5. The Predicted Value (log_{10} cells) vs. Observed Value (log_{10} cells) of *Salmonella dublin* in Stirred Fried Rice with Crab Meat by New Model (2)

between 25 and 43°C (Labbe & Garcia, 2001).

The comparison of predicted and observed growth values of *Salmonella amsterdam* and *Salmonella bangkok* shows that the observed values are lower than that of predicted values. The reason for this is that the model is developed for the growth of *Salmonella enteritidis,* which is a different subspecies from *Salmonella amsterdam* and *Salmonella bangkok* as shown in Table 2. On the other hand, the composition of predicted and observed growth values of *Salmonella dublin* showed that it fits well with the model. The reason for this is that *Salmonella enteritidis* and *Salmonella dublin* are the same subspecies but different in H antigen.

Conclusions

Mathematically, analysis and combined effects of environmental factors on growth of *Salmonella enteritidis, Salmonella amsterdam, Salmonella bangkok* and *Salmonella dublin* in

Table 2. Different Characters of Some *Salmonella subspecies*

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}
S. dublin	O ; 9 (D_1)	1, 9, 12 (Vi)	g, p

Source: Popoff and Le Minor (1997)

stirred fried rice with crab meat can be accomplished by altering the factors in the model. The result obtained from the CCD, fitting by a second-order model using a quadratic polynomial equation (1). After validation, the new model (2) is better than the former one. The new model accounts for 94.93, 79.36, 88.39 and 96.33 per cent accuracy for *Salmonella enteritidis*, *Salmonella amsterdam*, *Salmonella bangkok* and *Salmonella dublin*, respectively.

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