Development of a Predictive Program for *Salmonella Enteritidis* Growth in Ground Chicken and Liquid Egg Products

HIROSHI FUJIKAWA¹²*, AND MOHAMMAD ZAHER SAKHA²

¹Laboratory of Veterinary Public Health, Faculty of Agriculture Tokyo University of Agriculture and Technology 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan  
²Department of Applied Veterinary Science, the Graduated School of Veterinary Sciences, Gifu University

Received 29 December, 2012/Accepted 19 March, 2013

In this study, we developed a predictive program for *Salmonella Enteritidis* growth in ground chicken and liquid egg products at various temperature patterns. The ground chicken samples were sterilized chicken and raw chicken containing high and low levels of natural microflora, and the liquid egg products were pasteurized or unpasteurized. Microbial growth data published in our previous papers were used for prediction with our new logistic model. The program for the bacterial growth in those food materials was developed on a commercially available spreadsheet program. Users can instantly predict the *Salmonella* growth in those chicken and egg yolk products by inputting their temperature histories. The growth of natural microflora in the chicken products can also be predicted with the program. This program could be a useful tool to ensure the microbial safety of those materials with regards to *Salmonella Enteritidis* growth.

Key words : Predictive program / *Salmonella Enteritidis* / Ground chicken / Liquid egg / Logistic model.

Recently people tend to refrain from buying food products with food additives from the viewpoint of food safety. Also, it is becoming considerably important to produce and distribute food products under hygienically good conditions to decrease the possibility of microbial food poisoning outbreaks. Among various physicochemical factors related to food and food material such as hydrogen ion concentration (pH) and water activity, temperature is thought to be one of the most influential factors in controlling microbial growth. It is not desirable to add food additives, salt, or sugar to food for the purposes of microbial control from the viewpoint of taste or health.

Predictive microbiology models can be a tool to reduce microbial food poisoning outbreaks. Growth models can predict microbial growth under various environmental conditions. The modified Gompertz model, Baranyi model, and a three-phase model are growth models that are well-known worldwide, but there are some important problems with them (Gibson et al., 1987; Baranyi et al., 1994; Buchanan et al., 1997). We also developed the new logistic model with the original logistic model (Fujikawa et al., 2003 and 2004). The model successfully predicted bacterial growth at a variety of temperatures and its prediction performance was very similar to the Baranyi model (Fujikawa and Morozumi, 2005).

In predictive microbiology, expert computer programs have been developed for use in the food industry. When users input environmental data such as temperature, pH, and water activity into the program, it instantly predicts microbial growth and/or inactivation on the computer screen. The Pathogen Modeling Program (PMP) is one of the representative examples of these programs (http://pmp.arserrc.gov/PMPPHome.aspx). Combase is also a database program for predictive microbiology (http://wyndmoor.arserrc.gov/combase/). We have also developed some predictive programs for the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio parahaemolyticus* using a commercially available spreadsheet calculation program, Microsoft
Excel™. The predictive programs are available from the Japan Food Industry Center (http://www.shokusan.or.jp/haccp/news/index_18.html) (Fujikawa et al., 2006 and 2009).

However our predictive programs are for the growth of target microorganisms in culture media and pasteurized food (milk). PMP and Combase are also mostly based on the microbial growth data in culture media and sterilized food. However many of commercially available food and food materials are contaminated with various kinds of microorganisms. Growth of pathogens such as Salmonella and S. aureus in food is often suppressed by other contaminants due to microbial competition and/or antimicrobial substance production (Jay, 1992). Thus, for the food safety we need to estimate the growth of those harmful microorganisms in real food and food materials with contaminants as the second stage of predictive microbiology.

Among the pathogens related to food poisoning, Salmonella is one of the most important ones. It often contaminates food materials including eggs, meat, chicken, and vegetables (Jay, 1992). Especially, there have been worldwide, serious Salmonella outbreaks with serotype Enteritidis (Patrick, 2004). Thus, we recently reported the prediction of growth of S. Enteritidis and natural microflora in ground chicken and liquid egg products with the above model (Zaher and Fujikawa, 2011; Sakha and Fujikawa, 2012; Sakha and Fujikawa, 2013). In the present study, therefore, we report a predictive program developed for the pathogen’s growth in food materials.

Prediction of the growth of S. Enteritidis and natural microflora in those food materials at various temperature patterns in the present program was done with the new logistic model. The model is shown in equation (1).

\[
\frac{dN}{dt} = rN\left[1 - \frac{N}{N_{\text{max}}}\right]^{m}\left[1 - \left(\frac{N_{\text{max}}}{N}\right)^{n}\right] \tag{1}
\]

where \(N\) is the population of the organism (CFU/ml) at time \(t\) (h), \(r\) is the rate constant of growth (1/h), \(N_{\text{max}}\) is the maximum population (CFU/ml), and \(N_{\text{max}}\) is the initial population (CFU/ml). \(m\) and \(n\) are parameters related to the curvature of the deceleration phase and the period of the lag phase, respectively.

Parameter values in the model were estimated for prediction as follows. The \(r\) value at a given temperature was expressed with the square root model (McMeekin et al., 1992). The values for \(N_{\text{max}}\) which were dependent on temperature were expressed with a linear regression equation (Zaher and Fujikawa, 2011; Sakha and Fujikawa, 2012; Sakha and Fujikawa, 2013). The values for \(m\) and \(n\) were obtained from the data on the food materials.

The prediction procedures of this program are demonstrated as follows. When users predict the growth of Salmonella and natural microflora in the food materials with the program, they first choose the food material from the three ground chicken samples and two liquid egg products on the screen of the program. The ground chicken samples are the samples containing low and high levels of natural microflora at \(10^{3.7}\) and \(10^{5.8}\) CFU/g, respectively, and sterilized one (Zaher and Fujikawa, 2011). The liquid egg products in the program are pasteurized and unpasteurized products with natural microflora at \(10^{3.5}\) CFU/g (Sakha and Fujikawa, 2012).

Next, users input the temperature history of the food material on the screen. The program provides two ways to input the temperature history of the food. The first way is by manually inputting the temperature history on the screen. Users input the temperature and the period...
PREDICTIVE PROGRAM FOR SALMONELLA GROWTH

For each step of the temperature history; one example is shown in Fig. 1. The temperature history input in this manner gives an outline, as shown in the figure. The other way of inputting the data is by pasting the sequential data set of temperature and time recorded with a digital thermometer, which is shown afterwards in the present study.

Users then select the contamination level of the natural microflora of the food material. With the prediction button users can get the predicted growth curves of Salmonella and the natural microflora under that condition. An example for the growth prediction of Salmonella and the natural microflora in the ground chicken with the high level of the microflora is shown in Fig. 2. In this figure, the temperature history manually input in Fig. 1 is used for prediction. The program also demonstrates the populations of Salmonella and the natural microflora predicted at the time of interest; the predicted populations for Salmonella and the natural microflora after 12 hours of storage in this example are 104.6 and 108.0 CFU/g, respectively, in Fig. 2.

For the liquid egg products, the program can also show the growth prediction of Salmonella, as shown in Fig. 3. Salmonella growth prediction in liquid egg products with a high level of natural microflora. Predicted population of Salmonella at a given time (12 h) of storage is also shown below.

FIG. 2. Growth prediction of Salmonella Enteritidis and natural microflora in ground chicken with a high level of natural microflora. The upper and lower curves show predicted growths of the microflora and Salmonella, respectively.

FIG. 3. Salmonella growth prediction in liquid egg products with a high level of natural microflora. Predicted population of Salmonella at a given time (12 h) of storage is also shown below.

FIG. 4. Growth prediction of Salmonella Enteritidis and natural microflora in ground chicken with a low level of natural microflora. The recorded temperature data is pasted on the right-handed table. The upper and lower curves show the predicted growth of the microflora and Salmonella, respectively. Predicted populations of Salmonella and the microflora at a given time (20 h) of storage are also shown below.
ACKNOWLEDGEMENTS

This study was supported by Kieikai Research Foundation. The authors thank Dr. N. Sashihara and Ms. Y. Watanabe for their useful technical suggestions.

REFERENCES

Baranyi, J., and Roberts, T.A. (1994) A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol., 23, 277-294.

Buchanan, R.L., Whiting, R.C., and Damert, W.C. (1997) When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol., 14, 313-326.

Fujikawa, H., and Kai, A., Morozumi, S. (2003) A new logistic model for bacterial growth. J. Food Hyg. Soc. Japan, 44, 155-160.

Fujikawa, H., Kai, A., and Morozumi, S. (2004) A new logistic model for Escherichia coli at constant and dynamic temperatures. Food Microbiol., 21, 501-509.

Fujikawa, H., and Morozumi, S. (2005) Modeling surface growth of Escherichia coli on agar plates. Appl. Environ. Microbiol., 71, 7920-7926.

Fujikawa, H., Yano, K., Morozumi, S., Kimura, B., and Fujii, T. (2006) Development of a microbial growth prediction program at various temperature patterns. (in Japanese) J. Food Hyg. Soc. Japan, 47, 288-292.

Fujikawa, H., Kimura, B., and Fujii, T. (2009) Development of a predictive program for Vibrio parahaemolyticus growth under various environmental conditions. Bioccont. Sci., 14, 127-131.

Gibson, A.M., Bratchell, N., and Roberts, T.A. (1987) The effect of sodium chloride and temperature on the rate and extent of growth of Clostridium botulinum type A in pasteurized pork slurry. J. Appl. Bacteriol., 62, 479-490.

Jay, J.M. (1992) Modern Food Microbiology 4th ed. Chapman and Hall New York.

McMeekin, T.A., Ross, T., and Olley, J. (1992) Application of

FIG. 5. Salmonella growth prediction in the pasteurized liquid egg product. A predicted growth curve of Salmonella is described with the recorded temperature data that is pasted on the table. Predicted population of Salmonella at a given time (20 h) of storage is also shown below.
predictive microbiology to assure the quality and safety of fish and fish products. *International J. Food Microbiol.*, 15, 13-32.

Patrick, M.E., Adcock, P.M., Gomez, T.M., Altekruse, S.F., Holland, B.H., Tauxe, R.V., and Swerdlow, D.L. (2004) *Salmonella* Enteritidis infections, United States, 1985-1999. *Emerg. Infect. Dis.*, 10, 1-7.

Sakha, M. Z., and Fujikawa, H. (2012) Growth characteristics of *Salmonella* Enteritidis in pasteurized and unpasteurized liquid egg products. *Biocont. Sci.*, 17, 183-190.

Sakha, M. Z., and Fujikawa, H. (2013) Prediction of *Salmonella* Enteritidis growth in pasteurized and unpasteurized liquid egg products with a growth model. *Biocont. Sci.*, 18, 89-93.

Zaher, S.M., and Fujikawa, H. (2011) Effect of NM on the growth kinetics of *Salmonella* Enteritidis strain 04-137 in raw ground chicken. *J. Food Prot.*, 74, 735-742.