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Model on the microbial quality change of seasoned soybean sprouts for on-line shelf life prediction

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Abstract

The growth of aerobic bacteria on Korean seasoned soybean sprouts was modelled as a function of temperature to estimate microbial spoilage and shelf life on a real-time basis under dynamic storage conditions. Counts of aerobic bacteria on seasoned soybean sprouts stored at constant temperatures between 0 °C and 15 °C were recorded. The bootstrapping method was applied to generate many resampled data sets of mean microbial plate counts that were then used to estimate the parameters of the microbial growth model of Baranyi and Roberts. The distributions of the model parameters were quantified, and their temperature dependencies were expressed as mathematical functions. When the temperature functions of the parameters were incorporated into differential equations describing microbial growth, predictions of microbial growth under fluctuating temperature conditions were similar to observed microbial growth.

Keywords: Microbial growth model; Aerobic bacteria; Lag time; Growth rate; Fluctuating temperature

1. Introduction

Shelf life monitoring and quality control of chilled foods according to microbial criteria are becoming more important because there is increasing customer demand for high quality food and safety assurance. Online quality monitoring and decision support systems are needed to minimise microbiological spoilage and risk of food (Wijtzes et al., 1998; Koutsoumanisa et al., 2005; Yam et al., 2005). All methods of monitoring and evaluation should incorporate methods of relating storage and distribution conditions to changes in food quality. One such approach is to use a time-temperature integrator or indicator (TTI) in which colour changes are related to microbial growth rates (Taoukis et al., 1999). Recently, radio frequency identification (RFID) system consisting of a host computer system and RFID equipment (interrogator and transceivers) has been used to track food and transfer the information through distribution channels (Anonymous, 2004; Yam et al., 2005). Transceivers attached on the food packages as tags are capable of storing product information and of communicating this information to the interrogator or reader under the application program environment controlled by the host system. Information potentially to be stored and conveyed in RFID tags includes temperature, food quality and nutrition data. The application of TTI or RFID tags to real-time monitoring and estimation of food shelf life requires kinetic modelling techniques that relate environmental changes to food quality (Taoukis et al., 1999; Blackburn, 2000; Singh, 2000). Such kinetic models should be simple to manage and accurate enough to predict microbial counts under food supply chain conditions.

Many researchers have developed mathematical models of microbial growth under fluctuating temperature conditions (Baranyi et al., 1995; Van Impe et al., 1995; Vankerschaver et al., 1996; Taoukis et al., 1999; Bovill et al., 2000; Soboleva et al., 2001; Cheroutre-Vialette and Lebert, 2002; Huang, 2003; Nauta et al., 2003; Corradini and Peleg, 2005; Koseki and Isobe, 2005). Of these approaches, models that use differential equations to describe microbial growth are most easily applied to real-time prediction of microbial population density under changing external conditions.

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The differential equations are integrated with instantaneous acclimatization of time-dependent variables to simulate microbial quality at any given time in food supply chains. The microbial quality predicted on real time basis can be used as a criterion for shelf life management or logistic control. The dynamic growth model of Baranyi and Roberts (1994) with two differential equations has been widely used for predicting microbial growth under changing temperature conditions (Baranyi et al., 1995; Bovill et al., 2000; Shorten et al., 2004; Koseki and Isobe, 2005). Van Impe et al. (2005) extended the differential equations to incorporate the effects of nutrient exhaustion and metabolic waste products. Amezquita et al. (2005) combined the dynamic growth model with heat transfer model to predict the microbial growth in the centre of big size food product.

The aim of this study was to develop a simple microbial prediction model for determining the shelf life of Korean seasoned soybean sprouts, a popular product in the chilled food sector. Like most other seasoned side dishes in Korea, it is prepared by boiled cooking and mixing of spices/sauces. Because the product is usually packed and distributed without pasteurisation or sterilisation in hermetic package, it contains substantial microbial load, is usually stored, and marketed under chilled conditions. Aerobic bacterial growth is known to determine the sensory quality and shelf life of the product (Kim et al., 2003; Seo et al., 2006; Park et al., 2007), and it was used as an index quality for shelf life determination in this study. Although the growth of specific strains of spoilage bacteria on defined media is widely modelled for shelf life control of food (Sutherland, 2003), total aerobic bacterial growth was used as a general criterion for practical shelf life determination of a variety of chill-stored foods (Vankerschaver et al., 1996; Garcia-Gimeno and Zurera-Cosano, 1997; Lyhs et al., 2001; Blixt and Borch, 2002; Kim et al., 2003; Corbo et al., 2006).

2. Materials and methods

2.1. Seasoned soybean sprouts

Seasoned soybean sprouts were purchased from a shop in Masan, Korea. The seasoned sprouts were prepared by mixing 1000 g of steamed soybean sprouts with 24 g salt (Woil Salt, Ulsan, Korea), 19 g minced garlic, 18 g sesame oil (Ottogi, Ulsan, Korea) and 8 g sesame salt powder (Sea and Mountain, Busan, Korea). The product was transferred to the laboratory immediately after preparation and used for the storage experiment.

2.2. Storage of soybean sprouts under constant or fluctuating temperature conditions

In the first trial, seasoned soybean sprouts were stored under constant temperature conditions at 0, 5, 10 and 15 °C. Units of 100 g seasoned soybean sprouts were placed in 18.0 cm × 13.0 cm × 2.0 cm rectangular polystyrene trays. The tops of the packages were sealed with 12 μm thick linear low density polyethylene cling film (Clean Wrap, Busan, Korea). During the storage period, groups of three packages were removed at intervals for measurement of total aerobic bacterial counts on 20 g sample.

In the second trial, trays of seasoned soybean sprouts were subjected to various protocols in which temperatures were varied. Ten-gram samples of seasoned soybean sprouts were taken aseptically for measurement of total aerobic bacterial counts. Monitoring of temperature history was undertaken on the same food tray whose microbial count was measured with sampling a smaller amount of food from it.

In order to record the temperatures of seasoned soybean sprouts tray under real-time experimental conditions, we used a radio frequency identification (RFID) system consisting of two MICAz Mote development kits (MOTE-MPR2400), which were designed specifically for embedded wireless sensor networks (Crossbow Technology, San Jose, CA, USA). One kit with a temperature sensing module, attached on the tray, was used for measuring, collecting and transmitting temperature data in real-time via a wireless signal, and the other kit connected to a computer was used for receiving the temperature data. The thermistor temperature sensor used in the experiment can measure temperatures between −40 °C and 70 °C.

2.3. Enumeration of aerobic bacterial counts

For determination of microbial counts on seasoned soybean sprouts, 10 or 20 g in the sample were aseptically transferred to sterile Stomacher bags and diluted with 40 mL sterile 0.1% peptone water. Samples were then homogenised in a Stomacher (Lab-Blender, TMC International, Seoul, Korea) for two minutes at 300 strokes/min and 0.1 mL aliquots in triplicates were plated out directly or as 10-fold dilutions in 0.1% peptone water on Plate Count Agar (PCA; Difco Laboratories, Detroit, USA). The number of colony-forming units (cfu) per gram of sample was estimated after incubation for 72 h at 30 °C. Each experimental data point was based on single plate count. Mostly each enumeration consisted of three samples with triplicate plates except for the dynamic storage test with a sample tray only with triplicate plating.

2.4. Parameter estimation for the microbial growth model

The microbial growth model of Baranyi and Roberts (1994) was used to describe microbial growth mathematically.

$$\frac{dq}{dt} = \mu_{max} q \tag{1}$$

$$\frac{dN}{dt} = \mu_{max}\left(\frac{q}{1+q}\right)\left(1-\frac{N}{N_{max}}\right)N \tag{2}$$

where q is the normalized concentration of an unknown substance critically needed for cell growth and represents the physiological state of the cell population, μ_{max} is the maximum specific growth rate (1/day), N is the microbial count in cfu/g at time t, and N_{max} is the maximum cell density in cfu/g.

Even though there are only two parameters of μ_{max} and N_{max} in Eqs. (1) and (2), their solution requires two other parameters, q_o and N_o, which describe the initial physiological state and density of the microbial cells, respectively.

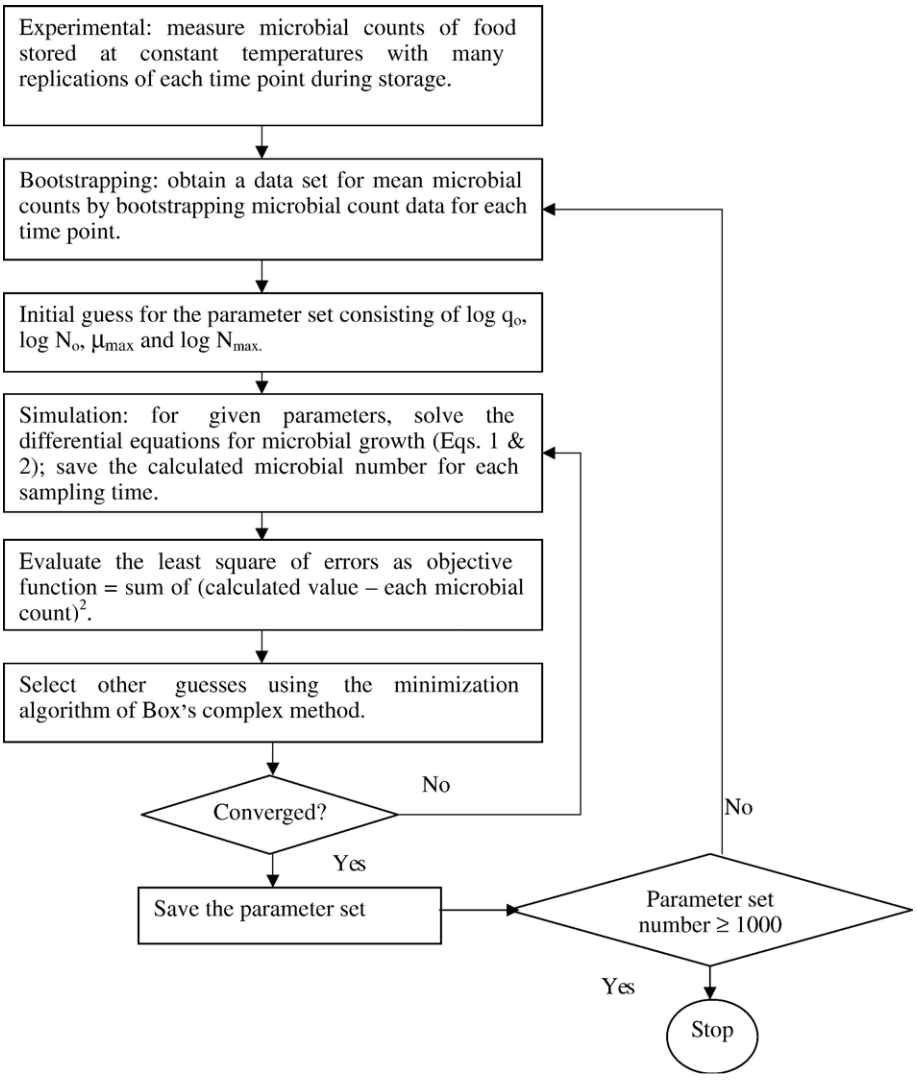

Fig. 1. Algorithm of microbial growth model parameter estimation by iterative simulation and optimisation.

The scheme for parameter estimation is outlined in Fig. 1, which was based on mean microbial counts obtained by bootstrapping method. For each experimental set at constant temperature, the accumulation of microbial cells was simulated for a particular set of parameters of log q_o, log N_o, μ_{max} and log N_{max} by solving differential Eqs. (1) and (2) using a fourth-order Runge Kutta method. The parameter set that minimised the sum of squares of errors between the simulated and observed microbial counts (in log (cfu/g)), was identified using a minimisation algorithm of Box's complex method (Saguy, 1983). The experimental data includes experimental error and variances of samples and plates even from the same batch production. The individual counts in this study contain sample variability and within-sample variability. Therefore as a way to take account of these variations, the data for mean microbial counts during storage were obtained by bootstrapping individual plate counts and used for the parameter estimation (Efron and Tibshirani, 1993; Schaffner, 1994). Estimates of mean microbial counts at sampling times were obtained from bootstrap replications generated by random sampling with replacement from the individual plate count data to construct a grouped set of microbial growth (Efron and Tibshirani, 1993), which was used for the parameter search. Bootstrapping and parameter estimation were repeated 1000 times to obtain data on the distribution of log q_o, log N_o, μ_{max} and log N_{max}. All of the computational procedures were written in Fortran code and run under a Digital Visual Fortran® environment (Digital Equipment Corporation, Maynard, MA, USA).

The average parameters of log q_o, log N_o, μ_{max} and log N_{max} determined in this study were also compared with those derived using the MicroFit® program (Institute of Food Research, Norwich, UK), which fitted Eq. (3) of the integrated form (Baranyi and Roberts, 1994, 1995) to the data set of average plate counts during storage.

$$\log N = \log N_o + \frac{\mu_{max}}{\ln(10)} \cdot A - \frac{1}{\ln(10)} \cdot \ln\left(1 + \frac{e^{\mu_{max} A} - 1}{10^{(\log N_{max} - \log N_o)}}\right) \quad (3)$$

where A is defined by $A = t + \frac{1}{\mu_{max}} \cdot \ln\left[\frac{e^{-\mu_{max} t} + q_o}{1 + q_o}\right]$.

Even though bootstrapping method may be combined with Eq. (3) to obtain the parameter distribution, it was not tried in this study which focuses on using differential equation forms (Eqs. (1) and (2)) to be applied to dynamic storage conditions.

2.5. Simulation of microbial growth under fluctuating temperature conditions

For simulation of microbial growth under fluctuating temperature conditions, Eqs. (1) and (2) were integrated numerically with the incorporation of a time-dependent maximum specific growth rate, μ_{max}. The temperature dependence of μ_{max} averaged from 1000 bootstrap replicate values was described by a square root model, Ratkowsky Eq. (4):

$$\sqrt{\mu_{max}} = b \cdot (T - T_{min}) \quad (4)$$

where T is the temperature (°C), b is a parameter ($day^{-1/2}\ °C^{-1}$), and T_{min} is the theoretical minimum temperature (°C) for growth estimated by extrapolation of the regression line to the temperature axis.

To solve Eqs. (1) and (2), the q value at the initial time (q_o), which is defined as the initial physiological state of the cells or the hypothetical concentration of critical substance at a bottle neck in the growth process (Baranyi and Roberts, 1994; Van Impe et al., 2005), should be supplied. In this study, it was assumed that the physiological state of the cell population is adjusted during the lag phase (t_{lag}), which is defined as the time required for the summed lag phase fraction (F_λ) to reach 1:

$$F_\lambda = \int_0^{t_{lag}} f(T)\mathrm{d}t = 1 \quad (5)$$

where f is the lag phase elapse fraction (inverse of lag time) corresponding to a certain temperature and is given as function of μ_{max} and q_o according to Baranyi and Roberts (1994) by following relation:

$$f = \frac{\mu_{max}(T)}{\ln(1 + 1/q_o(T))} \quad (6)$$

The dependence of μ_{max} and q_o on temperature is incorporated into Eq. (6) to reflect the effect of fluctuating temperature on the duration of lag time. This approach to calculating the lag phase is similar to that used by other researchers (Li and Torres, 1993; Huang, 2003; Nauta et al., 2003).

During the lag phase, microbial population density was assumed to stay at a constant level with adjustment of the physiological state, q. The initial q at the start of the lag phase was assumed to be adjusted to an averaged value of q_o as described by the following equation:

$$\log q_o = \frac{\int_0^{t_{lag}} \log q_o(T)\mathrm{d}t}{t_{lag}} \quad (7)$$

This assumption avoids dominant and determining effect of initial sharp temperature change on the microbial growth in the later storage with fluctuating temperature conditions. If q_o as function of temperature even for very short initial time is directly supplied to Eqs. (1) and (2) for dynamic microbial growth modelling, it will determine the microbial growth rate irreversibly in lag and exponential growth phases because the physiological state of microbial cells, q grows exponential from initial value q_o without decrease and bound as noted by Swinnen et al. (2004). It seem not relevant that q_o is solely determined by initial temperature which will change very soon.

In the lag and exponential phases, q was assumed to increase or build up, from the value determined by Eq. (7), according to Eq. (1). After the lag phase, log N at time t was obtained by numerical solution of differential Eqs. (1) and (2), starting from log N_o at the end of the lag phase. This scheme of simulation slightly differs from original growth model of Baranyi and Roberts (1994) in assuming q_o adjustment and no growth during lag phase duration obtained according to Eq. (5). It works to give the real-time estimation on microbial quality with little deviation from the original model, which is the aim of this study. The simulated values were compared to the experimental data for comparison and verification of the proposed model.

3. Results and discussion

3.1. Microbial growth model parameters

Fig. 2 shows the total aerobic bacterial counts on seasoned soybean sprouts that were stored under various isothermal conditions. Using the bootstrapping method for individual plate counts, the model parameters log q_o, log N_o, μ_{max} and log N_{max} were determined 1000 times to reveal their distributions (Fig. 3). The plots in Fig. 3 overlay bootstrap distributions of estimates of parameters with probability density functions of the fitted distribution (comparison of probability density) as one of the several goodness-of-fit plots. Identification and selection of the common distribution function for each type of parameter was based on the overall fit at four temperatures. These plots offer a visual comparison between the data and fitted distributions. No single function can explain the distribution of log q_o: the probability densities of log q_o at 0 °C and 5 °C are described by a logistic distribution function, while those at 10 °C and 15 °C are described by a BetaGeneral function. The probability density of μ_{max} has a logistic distribution at four

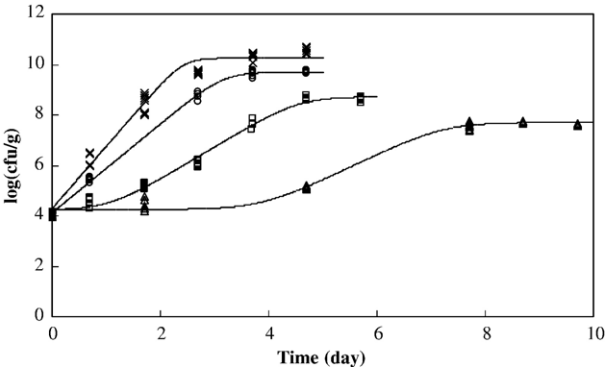

Fig. 2. Total aerobic bacterial counts on seasoned soybean sprouts stored at 0, 5, 10 or 15 °C. The solid lines are estimates derived using Eqs. (1) and (2) with an averaged parameter set. △: 0 °C; □: 5 °C; ○: 10 °C; ×: 15 °C.

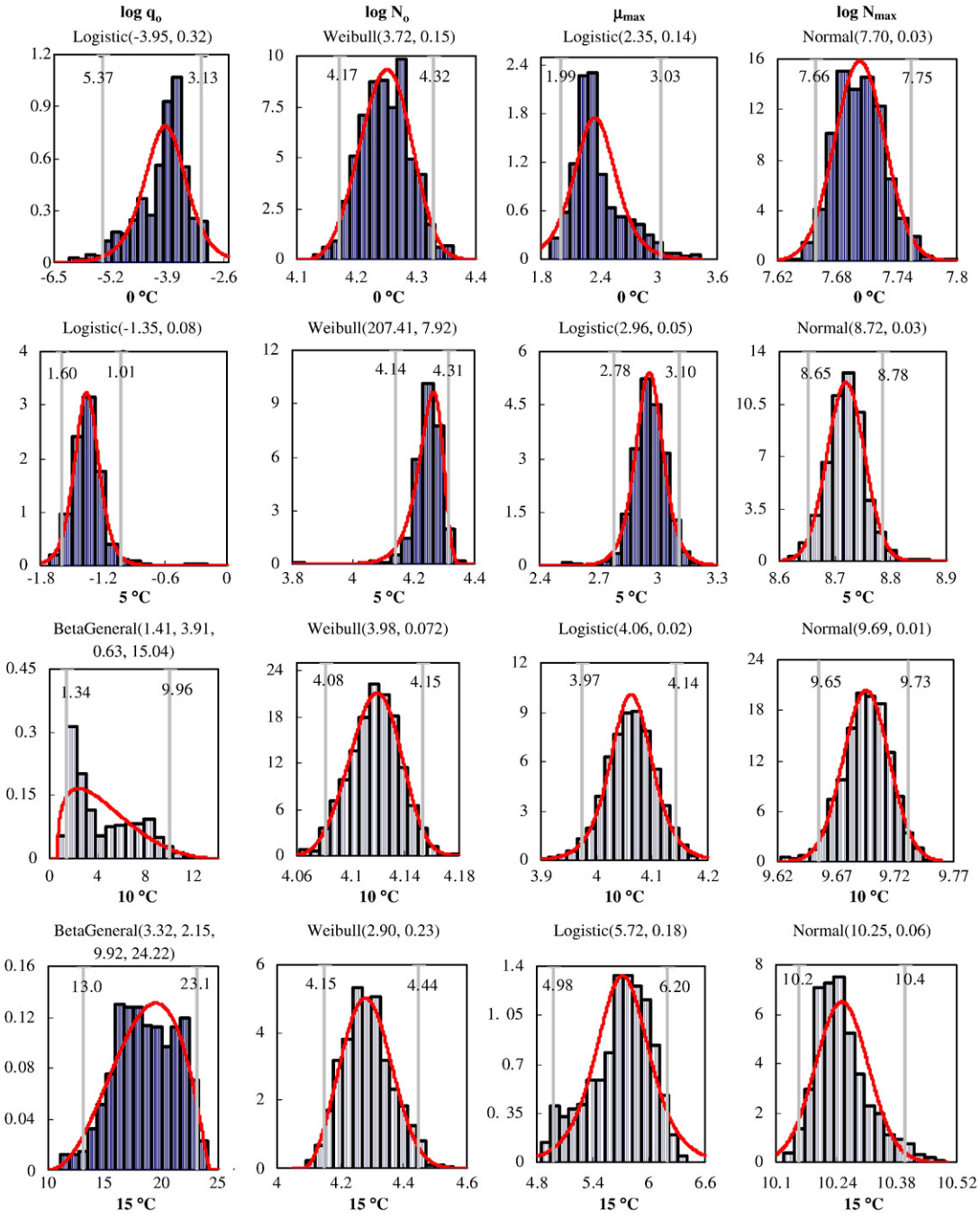

Fig. 3. Model parameter distribution for total aerobic bacteria counts on seasoned soybean sprouts stored at 0, 5, 10 or 15 °C. The unit of μ_{max} is in day^{-1}. The two vertical lines in the graphs depict 95% bootstrap confidence intervals. The distribution function parameters are given in brackets.

temperatures. Weibull and normal distributions describe log N_o and log N_{max}, respectively. Currently there are very limited data on the parameter distributions of microbial growth. Schaffner (1994) suggested that growth rate distribution of three microbial strains be between normal and Poisson. Poschet et al. (2003) represented the parameter distributions of microbial growth model of Baranyi and Roberts by normal distribution. It needs to be mentioned that the quality (data variability) and quantity (sampling frequency) affect the uncertainty and distribution of the model parameters with negligible influence on their mean value (Poschet et al., 2004). The fitted distribution functions in Fig. 3 may be used, in limited extent, to characterise the probability of the microbial growth parameters and produce a stochastic estimation of microbial growth by another further study as done by Poschet et al. (2003). Accumulation of more information on the parameter distribution in the future will improve the estimation.

Poschet et al. (2003) used Monte Carlo method, assuming lognormal distribution, to incorporate variation in experimental data and obtain the parameter distributions in the model of Baranyi and Roberts (1994). The present study of the parameter estimation is different from their work in that mean microbial count data were generated by bootstrap method using resampling from limited

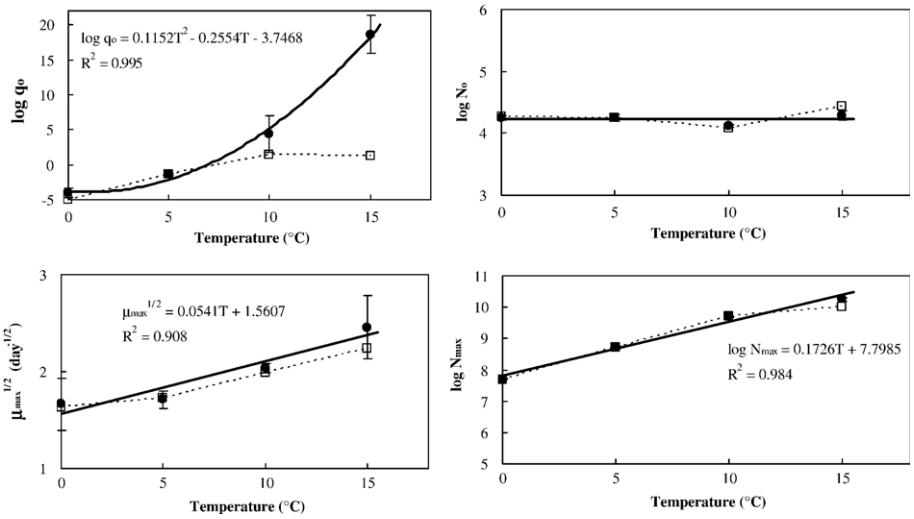

Fig. 4. Temperature dependence of the model parameters. ●: average value obtained from the algorithm illustrated in Fig. 1. □: value obtained from a MicroFit® regression of average microbial count data. Equations fitted to the values derived from the algorithm in Fig. 1 are shown in the graphs. Vertical bars indicate standard deviations of the results from algorithm of Fig. 1.

number of experimental data. The application of bootstrap method has advantage not to require any assumption on the distribution of microbial count data whose number is usually small and limited in the experiment. However, the scheme of parameter estimation is somewhat extended from the typical way to determine the confidence interval of model parameter by bootstrap method (Davison and Hinkley, 1997; Yafune and Ishiguro, 1999). The usefulness and limitation of the method adopted in this study is better to be compared to other methods of obtaining the parameter distribution, which is another topic of study.

Fig. 4 compares the average values of the determined parameters with the model parameters obtained with the MicroFit® program. There seems to be fairly good agreement between two methods except for q_o at 10 °C and 15 °C. For the MicroFit® program, lag time (t_{lag}) was substituted for q_o in Eq. (3) and determined by a nonlinear search. According to the model of Baranyi and Roberts, small differences in lag time very close to zero at high temperatures (10 °C and 15 °C) may cause high deviations in q_o when the lag time and the maximum specific growth rate were substituted into the following equation:

$$q_o = \frac{1}{\exp(t_{lag}\mu_{max}) - 1} \tag{8}$$

In other words, greatly different q_o values may result in similar lag times close to zero but produce very different microbial growth, significantly affecting the numerical solution of Eqs. (1) and (2).

The bootstrapped mean microbial count data set may also be used for the parameter estimation in Eq. (3) by using MicroFit® program or other nonlinear regression tools, which will provide another set of the model parameter distribution. Comparison of parameter distributions from different methods might give further information on the real parameter values and a guidance of their usage in microbial growth prediction. This study relied on the scheme of using differential Eqs. (1) and (2) in order to obtain the model parameters (Fig. 1), because those equations with the relevant parameters are thought to be useful for estimating microbial growth under dynamic conditions. Influence of search method on parameter distribution may be investigated thoroughly by another study later. This study was stayed under limited simple comparison to MicroFit® result based on average microbial growth data.

The temperature dependence of the obtained model parameters is shown in Fig. 4. While it is evident that log N_o is independent of the experimental conditions at hand, log q_o, μ_{max} and log N_{max} are strongly affected by temperature. The increase of maximum specific growth rate μ_{max} with temperature is reasonable as it is consistent with widely accepted microbial growth kinetics and could be described by a square root model, Ratkowsky equation:

$$\sqrt{\mu_{max}} = 0.0541 \cdot (T + 28.85) \tag{9}$$

Minimum growth temperature of −28.85 °C in Eq. (9) is very low compared to usual ones of spoilage bacteria, and is also noted with a low temperature influence on μ_{max} (McMeekin et al., 1993; Kim et al., 2003).

An increase in maximum cell density N_{max} with temperature has been reported (Zwietering et al., 1991; Koseki and Isobe, 2005). Fig. 4 shows that a simple linear relationship (Eq. (10)) can be used to describe the dependence of N_{max} on temperature for incorporation to Eq. (2).

$$\log N_{max} = 0.1726T + 7.7985 \tag{10}$$

The strong dependence of q_o on temperature, as shown in Fig. 4, does not seem to be consistent with the concept of an initial physiological state represented by a concentration of a critical growth substance divided by the Michaelis–Menten constant (Baranyi and Roberts, 1994, 1995). Based on this concept, samples used for different storage experiments would be expected to have the same or similar initial concentrations of critical substance irrespective of the ensuing storage temperature. Thus Baranyi et al. (1995) and

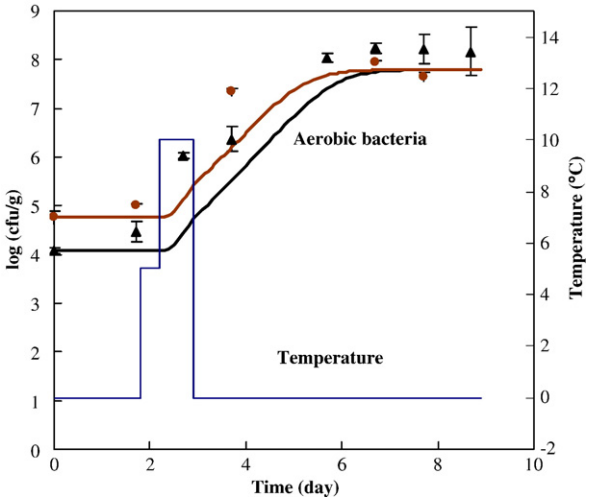

Fig. 5. Comparison between observed and simulated microbial growth on seasoned soybean sprouts stored under conditions in which temperature was varied in a stepwise manner. ●, ▲: experimental microbial data for two different samples. Storage-room temperature was applied to the simulation of microbial growth which was presented by thick solid lines for two different food samples. The thin line depicts temperature. Vertical bars indicate standard deviations of microbial data.

Koseki and Isobe (2005) adopted an averaged q_o value for predicting microbial growth under fluctuating temperatures, which caused some predictions to deviate from the experimental data, particularly under conditions involving high initial temperatures or sudden changes in temperature. The reasoning and assumption of identical q_o or initial physiological state for same culture have been questioned by the works of Alavi et al. (1999) and Mellefont and Ross (2003), which showed initial physiological state decreasing with larger negative temperature shift (from higher pre-incubation temperature to lower temperature) similarly to Fig. 4. The problem of using the parameter q_o for estimating lag time duration in the model of Baranyi and Roberts was discussed by Swinnen et al. (2004). Even though accurate estimations of initial physiological states of cell populations are required for accurate prediction of lag phase and microbial growth under dynamic conditions (Baranyi et al., 1995; Bovill et al., 2000), the problem was not resolved so far and needs further study for satisfactory answer.

From the results in Fig. 4 with the q_o value sharply increasing with temperature, we tried to elucidate the q_o value in a different way. The q_o value was assumed to be the relative readiness for adaptation for growth, which is more appropriate for higher temperatures. This concept was used for estimating microbial growth under dynamic temperature conditions. As discussed above, this peculiar phenomenal definition of q_o would have resulted from negative temperature shift and might need to be refined later for its versatility applicable to wide variety of environmental conditions. A quadratic equation was used to describe the temperature dependence of q_o for use in Eqs. (6) and (7):

$$\log q_o = 0.1152T^2 - 0.2554T - 3.7468 \quad (11)$$

3.2. Application of the model to dynamic temperature conditions

For reasons mentioned previously, the q_o was assumed to be adjusted during the lag phase of aerobic microbial growth: the time-averaged q_o determined by Eq. (7) was assumed to be ready for increased build up in the lag and exponential phases. When differential Eqs. (1) and (2) were solved with the incorporation of the temperature effect on q_o, μ_{max} and N_{max}, estimates of microbial counts under stepwise dynamic temperature conditions were similar to the experimental data (Fig. 5). Microbial growth of samples with two different microbial loads exposed to simple stepwise changes in temperature was generally well described by the model.

In the next step, more dynamic temperature changes were monitored with the RFID transceiver to simulate food distribution conditions and used as inputs to the computer program to estimate microbial growth on seasoned soybean sprouts. The estimated microbial growth with on-line monitored temperature changes was in fairly good agreement with the experimental data (Fig. 6). Some deviations might have been caused by sample variability and simplified model assumptions, as is the case for many other models (Wijtzes et al., 1998). In particular, the duration of the lag time under changing temperature conditions is known to be erratic and difficult to predict using currently available models (McMeekin et al., 2002; Bernaerts et al., 2004). More complex and rigorous treatment may help refine the proposed model and improve its predictive accuracy under dynamic temperature conditions. However, a simplified model with fewer parameters and tolerable error has been suggested and accepted as a useful and easy way of managing practical situations involving shelf life determination of food based on microbial criteria (McMeekin et al., 1993; Baranyi and Roberts, 1995). As long as the model is capable of prediction under dynamic conditions, different forms of primary and secondary models can be adopted and validated as useful tools for shelf life estimation (Peleg, 2006). Even with some limitations the

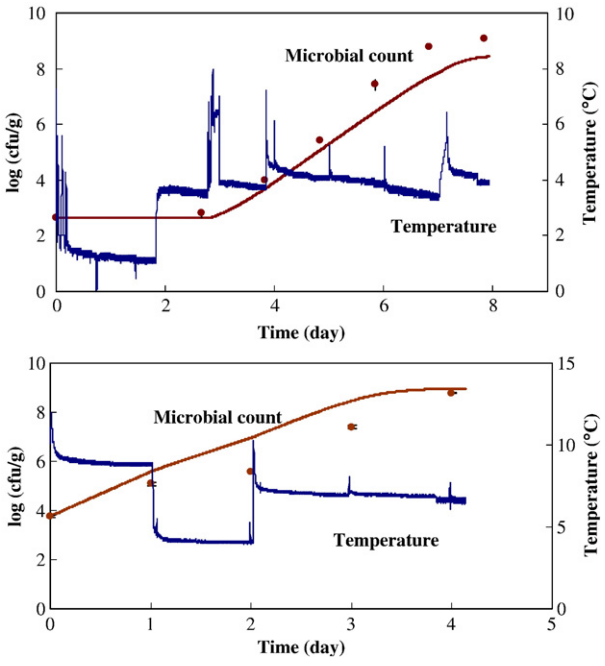

Fig. 6. Two sets of estimated microbial growth on seasoned soybean sprouts with on-line measurement of dynamically changing food temperatures. ●: experimental microbial data. Thick solid lines show estimated microbial growth. The fluctuating lines show temperature changes of food packages that were monitored by a RFID transceiver. Vertical bars indicate standard deviations of microbial data.

method proposed in this study has advantage to give on-line microbial quality of the food both in lag and exponential growth phases when its temperature is continuously monitored by RFID or any other devices.

Because recipes and processes vary between manufacturers, estimates for one processing factory may not be valid for another. However, this study showed that it is possible to predict the microbial quality of perishable food products produced under controlled conditions and thus to determine their shelf lives in real-time environments. Reliable confidence limits for estimates and a method for coping with variability in food and distribution channels are still needed (Moreau et al., 2005).

4. Conclusion

A method for estimating the parameters of the microbial growth model of Baranyi and Roberts was presented, and their distributions and temperature dependencies were quantified. The temperature functions of the parameters were incorporated into differential equations to predict microbial growth on seasoned soybean sprouts. The initial physiological state parameter depended on temperature and was assumed to be adjusted during the lag phase, which lasted until the accumulation of the temperature-dependent lag time consumption fraction reached 1.

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