A quasi-chemical model for the growth and death of microorganisms in foods by non-thermal and high-pressure processing

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Abstract

Predictive microbial models generally rely on the growth of bacteria in laboratory broth to approximate the microbial growth kinetics expected to take place in actual foods under identical environmental conditions. Sigmoidal functions such as the Gompertz or logistics equation accurately model the typical microbial growth curve from the lag to the stationary phase and provide the mathematical basis for estimating parameters such as the maximum growth rate (MGR). Stationary phase data can begin to show a decline and make it difficult to discern which data to include in the analysis of the growth curve, a factor that influences the calculated values of the growth parameters. In contradistinction, the quasi-chemical kinetics model provides additional capabilities in microbial modelling and fits growth-death kinetics (all four phases of the microbial lifecycle continuously) for a general set of microorganisms in a variety of actual food substrates. The quasi-chemical model is differential equations (ODEs) that derives from a hypothetical four-step chemical mechanism involving an antagonistic metabolite (quorum sensing) and successfully fits the kinetics of pathogens (Staphylococcus aureus, Escherichia coli and Listeria monocytogenes) in various foods (bread, turkey meat, ham and cheese) as functions of different hurdles (aw, pH, temperature and anti-microbial lactate). The calculated value of the MGR depends on whether growth-death data or only growth data are used in the fitting procedure. The quasi-chemical kinetics model is also exploited for use with the novel food processing technology of high-pressure processing. The high-pressure inactivation kinetics of E. coli are explored in a model food system over the pressure (P) range of 207–345 MPa (30,000–50,000 psi) and the temperature (T) range of 30–50 °C. In relatively low combinations of P and T, the inactivation curves are non-linear and exhibit a shoulder prior to a more rapid rate of microbial destruction. In the higher P, T regime, the inactivation plots tend to be linear. In all cases, the quasi-chemical model successfully fit the linear and curvi-linear inactivation plots for E. coli in model food systems. The experimental data and the quasi-chemical mathematical model described herein are candidates for inclusion in ComBase, the
developing database that combines data and models from the USDA Pathogen Modeling Program and the UK Food MicroModel.

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1. Introduction

The following information was presented at the 4th International Conference on Predictive Modeling in Foods held in Quimper, France, June 15–19, 2003 (Feeherry et al., 2003b).

We have recently introduced a unique “quasi-chemical” kinetics model for the growth and death kinetics of Staphylococcus aureus in shelf stable military bread (Taub et al., 2003). The basis for the quasi-chemical model derives from a simplified four-step chemical mechanism that includes a feedback step as a hypothetical analogue to the activity of an intercellular signalling molecule involved in the phenomenon referred to as quorum sensing (Dunny and Winans, 1999). These chemical reaction steps form a series of rate equations that generate a system of ordinary differential equations (ODEs) embodying the essence of the quasi-chemical model and imparting it with several distinctive features. The first characteristic distinguishing the quasi-chemical model from the mathematical models commonly used for predicting shelf life or non-thermal inactivation (e.g., Gompertz function, logistics equation, probabilistic models) is the capacity to model growth-decline kinetics (Taub et al., 2000a). In particular, the quasi-chemical model sequentially characterizes the four phases of the ordinary microbial lifecycle: lag, growth, stationary and death (Taub et al., 2000b). The second unique characteristic of the quasi-chemical model involves the method for estimating the maximum growth rate (MGR) from the slope of the inflection point of the sigmoidal growth curve. Using conventional models, the calculated value of the MGR depends on the judicious selection of the colony count data in the stationary phase, and the exclusion of colony count data that might indicate a decline (the onset of the death phase). The selection of the data used in fitting the function influences the calculated value of the MGR (Taub et al., 2003). The quasi-chemical model offers the ability to estimate a value of the MGR from the slope at the inflection point directly from the contiguous growth-death data, without having to subjectively select the stationary phase data or de-select data potentially indicating the kinetics of decline. This distinction results in differences in values for the estimated MGR using the quasi-chemical model or the Gompertz function for the same data set (Taub et al., 2003), although neither method can assume to be intrinsically superior or more reliable.

As described above, the quasi-chemical model is based on a schematic chemical reaction mechanism consisting of four reaction steps (Taub et al., 2003). The mechanism, in this case, involves a proposed antagonistic metabolite that is produced during the growth stage and acts as an intercellular signaling molecule (quorum sensing). This hypothetical signaling molecule acts on subsequent steps in the model and accelerates the death of cells. The ability of the quasi-chemical model to accurately fit microbial growth-death kinetics behavior in actual foods controlled by hurdles has been successfully demonstrated with S. aureus in bread as a function of incremental variations over a wide range of environmental conditions of water activity ($a_w$), pH and temperature (Feeherry et al., 2001, 2003a). Presently, we generalize the utility of the quasi-chemical model for characterizing microbial growth-death kinetics by exploiting its predictive capabilities in additional food substrates, for additional target pathogenic microorganisms, and for the addition of anti-microbial compounds to the food formulation. Specifically, we model the growth-death kinetics of S. aureus in intermediate moisture turkey meat, ham and cheese as functions of variations in $a_w$, pH and lactate content, and for the growth-death kinetics of Escherichia coli O157:H7 and Listeria monocytogenes in IM turkey meat. The success of the quasi-chemical model in fitting a variety of kinetics behaviors for a generalized set of pathogens, food substrates and
hurdles over a wide range of environmental conditions demonstrates its versatility and suggests that it may be sufficiently flexible to characterize the inactivation kinetics of pathogenic microorganisms using the emerging technology of high-pressure processing (HPP). HPP is a novel, non-thermal method of food preservation that retains higher levels of nutrients and sensory attributes such as texture, flavor and color. The commercial marketplace is growing steadily with food products preserved using this technology. Further development and growth of HPP as a food preservation technology, according to the 2003 IFT Summit (Heldman and Newsome, 2003), requires developing mathematical models that can accurately predict the inactivation of vegetative microorganisms and spores, then developing appropriate secondary models that accommodate the combined effects of P and T. We demonstrate the suitability of the quasi-chemical model for the high-pressure inactivation of E. coli (ATCC 11229) in a model food system (whey protein) over a range of systematic variations in conditions of pressure (P) and temperature (T).

2. Materials and methods

2.1. Sample preparation

Bread was prepared according to Military Specification MIL-B-44360A (11 March 1993) as described previously (Feeherry et al., 2003a; Taub et al., 2003). The a_w (0.79–0.90) was adjusted by varying the glycerol (0–12 wt.%) content of the dough. The pH of the bread was adjusted by varying the concentration (0–0.3 wt.%) of glucono-delta-lactone (GDL, Glucona America, Janesville, WI, USA) added to the dough. Raw turkey meat was cubed, infused with 1.75% NaCl, 0.05% Na-tripolyphosphate and 6.0% glycerol, and then packed in meat casings and cooked to an internal temperature of 71 °C. The cooked turkey was sliced into disks, and stacks of disks were then microwave-assisted freeze-dried to nominal a_w values (0.84≤a_w≤0.96). The samples were ground, dispensed in sterile Stomacher bags, frozen in dry ice, and then irradiated with a 60Co source with a dose level of 8.5–9.2 kGy over 135 min (STERIS Isomedix Services, Morton Grove, IL, USA) to eliminate competing microflora prior to inoculation. In some samples, the appropriate amount of 70% sodium lactate solution was added to create a 1%, 2% or 3% sodium lactate level in the brine for infusion into the raw turkey meat, and the preparation of the samples was completed as described above. A similar infusion process was followed to prepare glycerated ham samples, excluding the irradiation step. Process cheese product samples were purchased from a commercial vendor (Gamay Flavors, New Berlin, WI, USA). Whey Protein Concentrate 7504 (Calpro Ingredients, Corona, CA, USA) was dissolved in Butterfield’s phosphate buffer to produce 50% whey solutions that were inoculated for the high-pressure experiments.

2.2. Laboratory analysis of physical properties of food samples

The actual values of a_w, pH and moisture content were determined for the individual turkey meat, bread, ham, cheese and whey samples using standard laboratory analyses. Experimental values for a_w were measured using a Decagon CX-2 water activity meter at constant T=25 °C (Decagon Devices, Pullman, WA, USA), and determinations of pH were made using a glass spear-tipped Ross® combination electrode for foods connected to a Model 720A pH meter (Orion, Beverly, MA, USA). The moisture content of the individual samples was evaluated according to AOAC methods by determining the weight of moisture lost by 3–4 g samples dried under vacuum at 70 °C for 18–24 h.

2.3. Inoculum preparation

Bread, turkey, ham and cheese samples were inoculated to levels of approximately 10^4 cells/g food sample with stationary phase cultures of either S. aureus, E. coli O157:H7 or L. monocytogenes. The S. aureus cocktail comprised an equal mixture of A-100 (Natick Soldier Center, Natick, MA, USA), ATCC 14458 and 993 (Toxin Technology, Sarasota, FL, USA) and was prepared as described previously (Feeherry et al., 2003a). For high-pressure experiments, E. coli ATCC 11229 was prepared from stock cultures maintained on slants of nutrient agar (DIFCO, Sparks, MD, USA) that were transferred to nutrient broth (DIFCO) and incubated for 18 h at 35 °C. The culture was spread-plated on nutrient agar (0.3 ml culture on each of three agar plates, DIFCO) and incubated at 35 °C for
18–24 h. Harvesting the cells was done by scraping the nutrient agar surface with a sterile glass hockey stick and rinsing the nutrient agar plate with Butterfield’s phosphate buffer to produce a 10.0-ml stock solution of cells. The stock solution was diluted to yield a Klett54 colorimetry (Klett-Summerson Photoelectric Colorimeter, AH Thomas, Philadelphia, PA, USA) measurement of 125–135 Klett units. For the inoculation, 1.0 ml of the \textit{E. coli} ATCC 11229 stock solution was added to the whey protein solutions in 400-ml sterile stomacher bags (Model 400 bags, Seward, London, England, UK) that were vacuum-sealed (Röschermatic Vacuum Packaging Machine, Reiser and Canton, MA, USA). The sealed stomacher pouches were placed inside retortable pouches (3 Side Seal Pak, Kapak, Minneapolis, MN USA) and vacuum-sealed again to prevent leaks and microbial contamination of the high-pressure unit. High-pressure experiments were carried out using an EPSI high-pressure unit rated to 830 MPa (120,000 psi, Engineered Pressure Systems, Haverhill, MA, USA) with a temperature-controller and using water containing 5% Hydrolubic 120B lubricant (Houghton International, Valley Forge, Pa, USA) as the compression fluid. In general, compression heating raised the temperature only a few degrees and the temperature controller restored the target temperature within 3–10 min, depending on the conditions.

2.4. Data collection procedure

Bread, turkey meat, ham or cheese samples inoculated with \textit{S. aureus}, \textit{E. coli} O157:H7 or \textit{L. monocytogenes} were sealed in trilaminate pouches (Cadillac Products, Paris, IL, USA), incubated at constant temperature over the range of 15–40 °C. Samples were withdrawn incrementally for microbiological enumeration using a spread-plate technique on Baird-Parker agar supplemented with egg yolk and tellurite for \textit{S. aureus} and nutrient agar for \textit{E. coli} and \textit{Listeria} (DIFCO, Sparkes, MD, USA) and incubated at 35 °C for 48 h, then counted using a New Brunswick Colony Counter (New Brunswick Scientific, New Brunswick, NJ, USA).

2.5. Additional data collection procedure

50% aqueous whey protein solutions inoculated with \textit{E. coli} ATCC 11229 were subjected to high-pressure treatments for various times at various pressures and temperatures to acquire kinetics data. Following the high-pressure treatment, samples were diluted with Butterfield’s phosphate buffer and enumerated using a spread-plate technique. The diluted sample was spread-plated on Trypticase Soy Agar (DIFCO) with 0.6% added yeast extract (DIFCO). The plates were incubated at 35 °C and counted at 48 h using a New Brunswick Colony Counter (New Brunswick Scientific).

2.6. Data analysis and modeling

MATLAB software (The Mathworks, Natick, MA, USA) was used to integrate the ODE system using the solver ODE15s, and the optimization software package LSQNONLIN was used to carry out nonlinear least squares regression analysis of the microbiological data to evaluate best-fit values of rate constants for the colony count versus time data. Fitting the data was not entirely mechanical. In the case of the growth-death kinetics for the intermediate moisture foods, appropriate initial guesses of \(k_1\) through \(k_4\) were made, and then the ODEs were solved to determine calculated values of the colony counts (Taub et al., 2003). The calculated value was compared to the plate count data, and the rate constant values (\(k_1\) through \(k_4\)) were changed iteratively to minimize the discrepancy between the calculated and experimental values according to nonlinear least-squares procedures. In most instances, the data were smooth and the model fit the data remarkably well throughout the lag, exponential, stationary and death phases and adapted nimbly to small changes in the experimental parameters. Some data sets contained random noise and required large numbers of iterations to satisfy the convergence criteria. The randomness of the input data was reflected in the irregularity of the final estimates. In a few instances, final estimates of the rate constants could be noticeably affected by omitting one data point or by using different starting values. In the case of the high-pressure experiments, a similar procedure was carried out to model the observed inactivation kinetics, and equivalent solutions could be reached by modifying the values of the rate constants.

The detailed mathematical procedure for constructing a secondary model relating MGR, \(a_w\) and pH has been covered in detail (Taub et al., 2003), and will be described only briefly here. By assuming a linear relationship between the MGR, pH and \(a_w\) according to the relationship \[\text{MGR} = C_0 + C_1(a_w) + C_2(\text{pH}),\] regression
analysis of the data can estimate values for the linear coefficients \((C’s)\). Inserting the values of the \(C’s\) obtained from this least-squares analysis and setting \(\text{MGR}=0\) yields the relationship \(\text{pH}=11.48–7.30(\text{a}_w)\) that characterizes the various combinations of \(\text{pH}\) and \(\text{a}_w\) (at \(T=35^\circ\text{C}\)) that limit growth (see also Ratkowsky and Ross, 1995). Plotting this relation in the \(\text{pH–a}_w\) plane and the state of the observed microbial kinetics (as symbols for either growth or no-growth) at each set of experimental conditions produces a growth and a no-growth domain divided by the calculated boundary.

3. Results and discussion

3.1. Mathematical modeling of \(S.\ aureus\) growth-death kinetics in IM bread

The quasi-chemical model integrates the individual phases of the microbial life cycle into a series of chemical reaction steps with associated rate constants (Taub et al., 2003). The processes include: the activation of cells from the lag phase to the exponential phase of growth \((M \rightarrow M^*);\) a multiplication step \((M^* + A \rightarrow 2M^* + A);\) a termination step \((M^* + A \rightarrow M^{**} \rightarrow D);\) and a natural death step \((M^* \rightarrow D)\). The velocity \((v)\) of each step is related to the rate constant of the reaction \((k)\) and the concentration of the participating entities \(M, M^*, A\) and \(D\):

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Constant</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td>(M \rightarrow M^*)</td>
<td>(v_1 = k_1 M)</td>
</tr>
<tr>
<td>Multiplication</td>
<td>(M^* \rightarrow 2M^* + A)</td>
<td>(v_2 = k_2 M^*)</td>
</tr>
<tr>
<td>Sensitized death</td>
<td>(M^* + A \rightarrow M^{**} \rightarrow D)</td>
<td>(v_3 = (10^{-9}) k_3 M^* A)</td>
</tr>
<tr>
<td>Natural death</td>
<td>(M^* \rightarrow D)</td>
<td>(v_4 = k_4 M^*)</td>
</tr>
</tbody>
</table>

These equations form the basis for the set of ordinary differential equation system in which changes in the species concentrations with time proceed according to the velocities of the reactions that form or remove them:

\[
\frac{dM}{dt} = -v_1, \quad \frac{dM^*}{dt} = v_1 + v_2 - v_3 - v_4,
\]

\[
\frac{dA}{dt} = v_2 - v_3 \quad \text{and} \quad \frac{dD}{dt} = v_3 + v_4.
\]

The model robustly accommodates a diverse set of population-time profiles. For example, the model is sufficiently versatile to reflect changes in the growth-death kinetics of \(S.\ aureus\) in bread with systematic variations in \(a_w\) (Fig. 1), temperature (Fig. 2) and \(\text{pH}\).
(Fig. 3). Regression analysis with the model (smooth lines) shows good agreement with the experimental data points (symbols). Additionally, the model calculates death-only kinetics (inactivation) observed at sufficiently low values of $a_w$ ($\leq 0.79$), temperature (15 °C) and pH ($\leq 4.9$).

Fig. 2. Quasi-chemical modeling $S. aureus$ growth-death kinetics in bread at $a_w=0.90$, pH 5.23 and $T=15–40$ °C (temperature increments indicated in figure adjacent to the corresponding curve). See Fig. 5 in Taub et al. (2003).

Fig. 3. Quasi-chemical modeling of $S. aureus$ growth-death kinetics in bread at $a_w=0.86$, $T=35$ °C and in descending order as they appear in the figure with pH=5.38, 5.36, 5.31, 5.19 and 4.97 (or %GDL=0, 0.05, 0.1, 0.2 and 0.3, respectively). See Fig. 1 in Taub et al. (2003).
3.2. Modeling S. aureus kinetics in IM turkey, ham and cheese

Fig. 4 demonstrates the excellent agreement of the quasi-chemical model to S. aureus growth-death kinetics in turkey meat as a function of variations in $a_w$ at constant pH. The model also fits the observed death-only kinetics at $a_w \leq 0.84$. Similarly, the model successfully fits the growth-death kinetics and death-only kinetics of S. aureus in ham as a function of $a_w$ (Fig. 5), and the death-only observed kinetics in cheese as a function of $a_w$ (Fig. 6).

3.3. Modeling the pathogens S. aureus, E. coli O157:H7 and L. monocytogenes and anti-microbial lactate

Fig. 7 shows representative kinetics of the microorganisms E. coli, L. monocytogenes and S. aureus in turkey meat at pH=6.4 and $T=35^\circ C$. A growth-death kinetics scenario was selected for each microorganism (see Fig. 7) and, in each case, the quasi-chemical model (solid lines) successfully fits the observed data (symbols). The quasi-chemical model is therefore generalizable to the hurdle factors $a_w$, pH and $T$ for various IM food substrates and for several different pathogens. The addition of 0–3% lactate, an anti-microbial food additive, to turkey meat samples with constant $a_w=0.896$, pH=6.4, moisture content $\approx 58\%$ and incubation temperature ($35^\circ C$), and had pronounced effects on the growth of S. aureus (Fig. 8). Growth-death kinetics were observed at 0% and 1% lactate, but 2% lactate substantially diminished the growth kinetics (Fig. 8). A level of 3%, lactate acted as a bacteriostatic that inhibited growth completely over the entire sampling time (13 days).

3.4. Applying the quasi-chemical model to high-pressure inactivation of E. coli ATCC 11229

Aqueous solutions of whey protein in buffer samples were inoculated with E. coli ATCC 11229 to levels of approximately $10^8$ cells/ml and treated systematically for defined time intervals at various conditions of pressure over the range of 207–345 MPa (30,000–50,000 psi) and temperature over the range of 30–50 °C. The quasi-chemical model successfully fits the inactivation data over the entire span of experimental conditions (Figs. 9 and 10). As pressure
increases from 207 MPa (30,000 psi), the inactivation rate also increases and the kinetics profiles undergo a pronounced conversion from curved with an initial shoulder to decidedly linear behavior at 345 MPa (50,000 psi) at 50 °C (Fig. 9). Similarly, as the temperature increases systematically from 30 to 50 °C
at constant pressure of 310 MPa (45,000 psi), the inactivation rate of *E. coli* ATCC 11229 increases and the kinetics profiles also change from curvi-linear to linear (Fig. 10). In all cases, the quasi-chemical model was used to successfully fit the data, for both the curved and linear kinetics profiles.
3.5. Secondary modeling S. aureus growth in IM bread

As demonstrated above, the quasi-chemical model successfully fits (Figs. 1–8) the microbial growth-death kinetics of various pathogens (S. aureus, E. coli and L. monocytogenes), in various IM foods (bread, turkey meat, ham and cheese), and as functions of various environmental factors (a_w, pH, temperature and lactate content). Using this fitting procedure (see Section 2.6), the quasi-chemical kinetics model is also used to estimate values of the MGR in each of these cases. A secondary model can be constructed from the results of fitting the microbial kinetics curves with the

![Fig. 9. Quasi-chemical modeling of the high-pressure inactivation kinetics of E. coli ATCC 11229 in whey protein at T=50 °C and in descending order in the graph of P=207, 242, 276, 311 and 345 MPa (corresponding to 30, 35, 40, 45 and 50 kpsi), as indicated in the figure.](image)

![Fig. 10. The effect of temperature (T=30–50 °C, as indicated in figure) on high-pressure inactivation plots of E. coli ATCC 11229 in whey at 310 MPa (45 kpsi) using the quasi-chemical model.](image)
The quasi-chemical model is a unique and versatile mathematical model for characterizing the kinetics of microbial behavior in foods. The model describes with decent fidelity growth, growth-death and death-only (inactivation) scenarios of microbes, sensitively accommodating small changes in the food environment over a wide range of experimental conditions. The quasi-chemical model has been extended to account for a general set of hurdles ($a_w$, pH, $T$ and the presence of the anti-microbial additive lactate), pathogens and food substrates. In addition to evaluating the MGR for the microbes in these conditions, the quasi-chemical model was also used to construct a secondary model by interrelating the MGR with $a_w$ and pH for the growth of $S. aureus$ in bread. This secondary model calculated a boundary line that divided combinations of $a_w$ and pH into domains of “growth” and “no-growth” and the data (including two arbitrary test points) showed excellent concordance with the model. The versatility of the quasi-chemical model has been demonstrated by its application to the novel non-thermal food processing technology of HPP. In this case, the high-pressure inactivation kinetics of $E. coli$ ATCC 11229 has been successfully characterized with this unique and advanced model. Specifically, the quasi-chemical model was fitted to the non-linear inactivation profiles observed at relatively low combinations of $P$ and $T$ and to the observed linear inactivation kinetics traces at higher combinations of $P$ and $T$. This model will continue to be refined and applied to HPP inactivation kinetics as we carry out experiments.
exploring tailing and other non-linear phenomena, spore inactivation kinetics and comparisons with other non-linear models (e.g., the Weibull distribution). The capabilities of the unique and advanced quasi-chemical model will be exploited for incorporation of the data described above and for the quasi-chemical model itself into the proposed ComBase program that combines the USDA Pathogen Modeling Program and the UK Food MicroModel that is currently in development and soliciting proposals.

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References


