System dynamics: A Complementary Tool for Predictive microbiology

Gabriela Guadalupe Gastélum-Reynoso

Departamento de Ingeniería Química y Alimentos, Universidad de las Américas Puebla. Cholula, Puebla, 72820. MEXICO +52 (222) 229-2126 gabriela.gastelumro@udlap.mx

Enrique Palou

Centro para la Educación de las Ciencias, Ingenierías y Tecnologías, Universidad de las Américas Puebla. Cholula, Puebla, 72820. MEXICO +52 (222) 229-2658 <u>enrique.palou@udlap.mx</u>

Aurelio López-Malo Departamento de Ingeniería Química y Alimentos, Universidad de las Américas Puebla. Cholula, Puebla, 72820. MEXICO +52 (222) 229-2409 aurelio.lopezm@udlap.mx

Abstract

One of the most important concerns for food industry is safety. Predictive microbiology is the application of mathematical models to describe microbial behavior in order to prevent food spoilage as well as food-borne illness. Because of complexity of microbial behavior and food systems, predictive microbiology presents some limitations. System dynamics could be a useful alternative and complementary tool to model and predict microbiological behavior in foods while providing a graphical interface and structures linked with a series of equations, to clarify and improve quantitative predictive microbiology descriptions.

Key words: Predictive microbiology, Food Microbiology, Food Safety

Introduction

One of the most important concerns for the food industry is safety. The modern concept of food safety has been influenced first by consumer's demand for fresher and healthier foods and second by the industry, producing safer products lowering the risk of the presence of food-borne pathogens and spoilage microorganisms in food products (Martínez et al., 2005).

Microbial ability to survive or die depends on product composition and combinations of a variety of food factors (pH, water activity and temperature among others). These food factors act as barriers (or hurdles) to the microorganism. The goal in terms of food safety therefore is to prevent the growth and/or toxin production of the pathogenic and spoilage microorganisms by determining the optimum conditions of food preservation processes (López-Malo, Guerrero and Alzamora, 2000).

There are several tools to assure food safety. Storage stability tests, microbial challenge tests and predictive microbiology. Predictive microbiology is the application of mathematical models to describe microbial behavior during food processing (Alzamora, Tapia and López-Malo, 2000; Betts and Everis, 2005).

Because of the complexity of microbial behavior and food systems, predictive microbiology presents some limitations. The most relevant limitation for the purposes of this paper is that existing microbial modeling tools are not integrated. Furthermore, mathematical models are simplifications of complex microbial and biochemical processes; in some cases not every important variable or factor that is affecting the system is included (Buchanan and Whiting, 1997); and finally, some aspects like fluctuating conditions (microbial behavior food environment and food processing steps) are not usually considered (Lebert and Lebert, 2006). System dynamics could be an alternative approach for describing simulating and predicting microbial food models since it is a methodology that studies complex feedback systems, within an integrated point of view, using simple algebraic equations and structures that function as building blocks for more complex models.

The aim of this paper is to suggest system dynamics as an alternative and complementary approach to model and predict food microbial behavior. In this way, the rest of the paper is organized in three sections: First, a brief review of predictive microbiology and its limitations are presented, while introducing system dynamics as an alternative and complementary approach to model microbiological processes in foods. Next, there is an example of a work in progress to model bacterial inhibition by heat treatment, using system dynamics. The paper ends with suggestions for further work.

Review of the Approaches

PREDICTIVE MICROBIOLOGY

In respond to consumer's demand for fresher, testier and healthier foods, food industry has developed new alternatives such as hurdle technology. This methodology combines several factors or hurdles (temperature, antimicrobials, a_w reduction, etc.) in order to ensure food quality and safety. The hurdles applied are milder than in traditional techniques (pasteurization, canned foods). Therefore, there is an increased risk of growth of pathogenic or spoilage microorganisms (Gould, 1995). In this way storage stability tests (SST), microbial challenge tests (MCT) along with predictive microbiology are common tools to predict and assure food safety. SST and MCT are laboratory experiments performed with products and microorganisms under controlled conditions. These experiments along with predictive microbiology provide information about the effects of different factors influencing safety of food products.

Predictive microbiology is the application of mathematical models to describe and predict microbial growth, survival, and/or inactivation as well as biochemical processes under specific time and conditions. Usually, microorganisms are grown under certain controlled factors (conditions) such as variations on pH, water activity (a_w), temperature, and antimicrobial concentration, among others. The results of their responses (biological parameters) are fitted into mathematical equations. Such models can be used to predict microbial responses under new conditions, within tested ranges of studied factors or its combinations (Betts and Everis, 2005; Alzamora, Tapia and López-Malo, 2000; Whiting and Buchanan, 1994; Lebert and Lebert, 2006).

McMeekin and his colleagues (1992) proposed some guidelines for developing effective models. Some of these guidelines consist of fitting precision, capacity to predict combinations of factors that have not been proven, incorporation of every outstanding factor, minimum number of variables, specification of the error term, parameters having biological meaning and real values, and reparameterization to improve statistical properties (McMeekin, Roos and Olley, 1992; Martínez et al., 2005).

Predictive microbiology models are classified in three levels. <u>Primary level models</u> describe the changes in microbial population over time (colony forming units (CFU)/ ml, toxin production, etc.) and provide information about microbial behavior such as lag phase duration. <u>Secondary level models</u> describe the changes in parameters of primary level (e.g. CFU/ml) when environmental factors change (pH, a_w, temperature, among others). <u>Tertiary level models</u> are interfaces, which convert the primary and secondary level models in friendly software where users can input environmental factors for different microorganisms and obtain a prediction of microbial population parameters. *Pathogen Modeling Program, Food MicroModel* and *Seafood Spoilage Predictor* are examples of available tertiary level models.

Models help understand and predict the behavior of microorganisms for specific conditions. Nevertheless, in some cases there is not an accurate fit for extrapolation to real life conditions. Then validation of a model becomes necessary before it is used to make food safety decisions. The validation process is done considering biological knowledge of the system and statistical tools. Once models are validated and users are aware of the limitations of the models, they are useful tools to obtain information and make decisions for the following situations (Alzamora, Tapia and López-Malo, 2000; Buchanan and Whiting, 1997):

- 1. Prediction of safety: Estimate the risk of growth or survival of pathogens during food processing.
- 2. Quality control: Improve systems like HACCP (Hazard Analysis of Critical Control Points) to ensure food safety.
- 3. Product development: Redesign processes and recipes, set priorities in product design and evaluation.
- 4. Data analysis and laboratory planning: The model could save resources, time and money.
- 5. Risk assessment models: Evaluate the probability that a food could cause foodborne illness.

Limitations of predictive microbiology

Even thought predictive microbiology models are widely used when correctly validated, they have several limitations because of the complexity of microbial behavior and food systems:

- Mathematical models are simplifications of complex biochemical processes and in some cases not every important variable or factor that is affecting the system is included in the model (Alzamora et al., 2005; Buchanan and Whiting, 1997).

- Usually, models are not designed for the same conditions in which microorganisms exist in food systems (biofilms, starved and unknown nutrients among many others), since the majority of the data to generate the predictive model are derived form brothbased experiments. It is known that bacterial pathogens are more resistant in real food products than in broth cultures (Alzamora et al., 2005).

- Most of the models describe changes of microbial behavior for homogeneous populations; nevertheless, competition among microorganisms affect the food environment (Lebert and Lebert, 2006).

- Some models make a good description of linear relationships; but when more than one factor is involved, reparameterization of the model becomes necessary. Response surface models can describe microbial growth when more factors are involved, however it is necessary to assume that biological parameters are independent and only works well within variable ranges for which the experiment was designed, that means extrapolation is not possible (Alzamora et al., 2005; Lebert and Lebert, 2006).

- On the other hand, accuracy of the model depends on the quality of the data, the standardization of the experimental and statistical methodology and the statistical measures of uncertainty. Literature or industry data usually lacks these types of information (Alzamora et al., 2005; Buchanan and Whiting, 1997; Marks, 2008).

- Finally, several inaccurate predictions are because of the models predict microbial growth or inactivation as an isolated event. It is necessary to integrate dynamic bacterial behavior (predictive microbial models), fluctuating conditions within the system (thermodynamics of the food product) and processing models (mass and heat transfer), in order to have the whole picture regarding the microbiological safety of foods. New approaches like integrated modeling (Lebert and Lebert, 2006) and Van Impe model (2005) describe microbial growth evolution as a self-limiting process, and consider both, physico-chemical food properties as well as mass and heat transfer during food processing as can be seen in Fig.1 (Marks, 2008). Although the effort to predict food microbial behavior as a complex event exists, the solutions are few, since models have been designed for specific conditions and objectives; then it is necessary to separately validate and to verify assumptions about the system before the integration model can be utilized (Lebert and Lebert, 2006).

For the reasons above mentioned, it is very important to explore and research other possibilities to integrate and validate food-processing variables. Since system dynamics

is a methodology that studies complex feedback systems, could be an alternative and complementary approach to understand, model, and predict microbial behavior in foods.



Fig. 1. An integrated modeling approach to predict bacterial behavior (Lebert and Lebert, 2006).

System dynamics as a complementary tool for Predictive microbiology

Food microbiology deals with complex feedback systems such as growing, surviving and dying populations of microorganisms, which can be found in culture media, laboratory-model systems or food products. Some microbiologists describe food as complex ecosystems that are conformed by the environment represented by food components and the microorganisms that can grow in the food product (Montville, 1997). Microbial ability to grow, survive or die (level variable) depends on the rate variables of growth and deaths (microorganism behavior), which in turn depend on both, inhibition factors related to food physico-chemical properties (pH, a_w, etc.) and extrinsic factors that are part of process engineering (temperature, ultrasound, natural antimicrobials, among others). On the other hand, bacteria tend to come back to its normal status (homeostasis). These homeostasis mechanisms involve a considerable energy cost for the microorganism that is to say, bacteria will use the energy for cell regeneration instead of cell reproduction and eventually the microorganism will die (Fig. 2). In consequence if bacterial homeostasis is perturbed by preservation hurdles, the microorganism will not multiply or even die before its homeostasis is repaired (Gould, 1995). Combinations of factors could promote dominance of either positive or negative feedback loops (Fig. 3).



Fig. 2. Energy available for bacterial metabolism and homeostasis.



Fig. 3. Hurdle technology and system dynamics building blocks.

Example: Bacterial Thermal Inactivation

Fig. 4 represents an example of a work in progress for bacterial inactivation by heat treatment, which is applied in order to prevent microbial growth and reproduction enhancing food safety (Valdramidis et al., 2006). The number of bacterial cells (stock 1) depends on cell viability (stock 2); when cell viability decreases, bacterial accumulation decreases as well. Cell viability is affected by the rate variables: *Energy* (for bacterial homeostasis) and *temperature goal* (heat treatment). The flows that affect bacterial accumulations are growing and dying. The flows affecting cell viability are reinforcing cell viability, deteriorating viability by natural death, deteriorating viability by heat treatment, and regenerating cell viability. The clouds represent the boundaries of the system.



Fig. 4. Biological system dynamics model: an example of a bacterial thermal inactivation process.

Temperature is one of the most important food preservation factors, since it affects microbial growth and reproduction rates. The optimal growth temperature for a microorganism is the one that allows the maximum growth rate. Below the minimum temperature and above the maximum temperature, microbial growth does not exist due to changes in bacterial metabolism. The optimal growth temperature for mesophilic bacteria is between 25°C and 40°C.

In this example, the model predicted the number of bacteria, deaths, births and cell viability (Figs. 5 and 7) as well as the average cell viability and the effective death and reproduction rates (Figs. 6 and 8). The experiment was carried out for two different conditions. The temperature of the first tested condition was 25°C, which corresponds to low optimal growth temperature for mesophilic bacteria. The temperature of the second tested condition was 70°C, temperature used for pasteurization. The time of the experiment duration was 10 min and maximum death rate and minimal viability remained constant for both tested conditions.

At 25°C, experiment results were as expected. The number of bacteria and viability were high and they correspond with the birth values, which were slightly higher than the deaths at the end of the treatment (10 min). After the treatment, the births are still growing (Fig. 5) because the reproduction rate and average cell viability are both above the death rate (Fig. 6).



Fig. 5. Effect of heat treatment on system variables at 25°C: 1. Bacterial number, 2. Deaths, 3. Births, and 4. Cell viability.



Fig. 6. Effect of heat treatment over the system variables at 25°C: 1. Effective death rate, 2. Cell viability, and 3. Effective reproduction rate.

When heat treatment was applied (70°C), bacterial inhibition was observed. In the first minute, births begin to decrease; the same happened with cell viability and the number of bacteria for three minutes. On the other hand, the deaths reached their peak at the fifth minute (Fig. 8). Average cell viability decreased in a way that gave no chance for the bacteria to increase its effective reproduction rate, yielding to total bacterial death before the heat treatment ended (Fig. 9).



Fig. 7. Effect of heat treatment on system variables at 70°C: 1. Bacterial number, 2. Deaths, 3. Births, and 4. Cell viability

Fig. 8. Effect of heat treatment on system variables at 70°C: 1. Effective death rate, 2. Cell viability, and 3. Effective reproduction rate.

Final Remarks

Foods are complex feedback systems. Predictive microbiology has developed several mathematical models to describe and predict food microbial behavior within these systems in order to achieve food safety while enhancing food quality. System dynamics seems to be a complementary approach to model and predict microbial behavior in food systems within an integrated point of view, since complex behaviors can be described with simple structures such as the casual-loop and stock-and-flow diagrams.

Furthermore, the structural diagram can be quantitatively described with simple algebraic equations using friendly software. Therefore, further work is underway to model biological parameters of a specific food system by means of predictive microbiology models and compare the results with those obtained by means of system dynamics models.

References

- Alzamora, S.M., S. Guerrero, P. Viollaz and J. Welti-Chanes. 2005. Experimental protocols for modeling the response of microbial populations exposed to emerging technologies: Some points of concern. In *Novel food processing technologies*, edited by G. Barbosa-Cánovas, M.S. Tapia and P.M. Cano. Boca Ratón, Florida: CRC, Press.
- Alzamora, S.M., M.S. Tapia and A. López-Malo. 2000. Overview. In *Minimally* processed fruits and vegetables. Fundamental aspects and applications, edited by G. Barbosa-Cánovas and M.S. Tapia. Gaithersburg, Maryland: Aspen Publishers, Inc.

- Betts, G. and L. Everis. 2005. Modeling systems and impact on food microbiology. In *Novel food processing technologies*, edited by G. Barbosa-Cánovas, M.S. Tapia and P.M. Cano. Boca Ratón, Florida: CRC, press.
- Buchanan, R.L. and R.C. Whiting. 1997. Predictive modeling. In *Food microbiology fundamentals and frontiers*, edited by M.P. Doyle, L.R. Beuchat and T.J. Montville. Washington, D C: ASM Press.
- Gould, G. W. 1995. *New Methods of Food Preservation*. London, UK: Blackie Academic & Professional.
- Lebert, A. and I. Lebert. 2006. Quantitative prediction of microbial behaviour during food processing using an integrated modeling approach: A review. *International Journal of Refrigeration* 29:968-984.
- López-Malo, A., S. Guerrero and S.M. Alzamora. 2000. Probabilistic modeling of Saccharomyces cerevisiae inhibition under the effects of water activity, pH, potassium sorbate concentration. Journal of Food Protection 63 (1):91-95.
- Marks, B.P. 2008. Status of microbial modeling in food process models. *Comprehensive Reviews in Food Science and Food Safety* 7 (1):137-143.
- Martínez, A., M. Rodrigo, D. Rodrigo, P. Ruiz, A. Martínez and M.J. Ocio. 2005. Predictive microbiology and role in food safety systems. In *Novel food processing technologies*, edited by G. Barbosa-Cánovas, M.S. Tapia and P.M. Cano. Boca Ratón, Florida: CRC Press.
- McMeekin, T.A., T. Roos and N. Olley. 1992. Application of predictive microbiology to assure the quality and safety of fish and fish products. *International Journal of Food Microbiology* 15:13-32.
- Montville, T.J. 1997. Principles which influence microbial growth, survival and death in foods. In *Food microbiology. Fundamentals and frontiers*, edited by M.P. Doyle, L.R. Beuchat and T.J. Montville. Washington, D.C.: ASM Press.
- Valdramidis, V.P., A.H. Geeraerd, K. Bernaerts and J.F. Van Impe. 2006. Microbial dynamics versus mathematical model dynamics: The case of microbial heat resistance induction. *Innovative Food Science and Emerging Technologies* 7 (1-2):80-87.
- Van Impe, J.F., F. Poschet, A.H. Geeraerd and K.M. Vereecken. 2005. Towards a novel class of predictive microbial growth models. *International Journal of Food Microbiology* 100:97-105.
- Whiting, R.C. and R.L. Buchanan. 1994. Microbial modeling. *Food Technology* 48 (6):113-119.