Contamination and Changes of Food Factors during Processing with Modeling Applications - Safety Related Issues

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ABSTRACT

Chemical and microbiological contaminations of food during processing and preservation can result in foodborne illness outbreaks and/or poisoning. Chemical contaminations can occur through exposure of foods to illegal additives, pesticides and fertilizer residues, toxic compounds formed by chemical reactions, and are easier to control than illnesses caused by microorganisms. In general, chemical reactions in food can be described using the first-order kinetic models. Food quality factors related to nutrition, color, texture, *etc*. are typically affected by temperature, pH, moisture content, as well as microbial growth. However, modeling the growth and inactivation of microorganisms is much more difficult and complex than that for chemical reactions. If harmful microbes are not eliminated during processing, their survival and growth may cause spoilage and foodborne illness. Food safety intervention technologies such as microwave heating, and modified packaging were discussed, as well as issues related to cross-contamination of foods during processing. Microbial food safety can be enhanced by the development of growth and inactivation modeling tools, world-wide data sharing, and collaboration in which the Pathogen Modeling Program (PMP) and ComBase can be utilized.

Key words: Foodborne pathogens, antimicrobial agents, MAP, microwave, modeling

INTRODUCTION

Food processing is common in the food supply chain to ensure safety, preserve food qualities and extend shelf life. Food property changes and contaminations may occur during the process operations and storage. Important food factors for the safety concerns may include microbial and chemical aspects. Microbes can be further divided into two categories, i.e. the good and bad bugs. The good microorganisms have been applied in the fermentation, for example, to produce flavors and spirits for centuries. The bad microorganisms including foodborne pathogens, e.g. *Listeria monocytogenes*, *Escherichia coli* O157 : H7, *Salmonella,* Hepatitis A, Norovirus and *etc*., are still causing human illnesses in recent years[(1)]. Contamination of harmful chemicals during processing usually can be predicted or prevented. Sources of potential chemical contamination sources are ingredients, additives (illegal, pesticide, or fertilizer residuals) and *etc*. (water contamination). Chemical reaction may generate carcinogenic compounds, e.g. acrylamide[(2)] during high-temperature baking or frying or heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs)[(3)] from charred or grilled foods in the open air. Microbial contamination is more complicated than chemical which involves living microorganisms in a nutrient-rich medium (foods). In this paper, the foodborne pathogens will be discussed further.

Three notorious foodborne pathogens caused major human illness are *L. monocytogenes, E. coli* O157 : H7, and *Salmonella* spp., in which *L. monocytogenes* is at the 'zero tolerance' status for ready-to-eat foods in USA. When microbes grow in foods, some toxic compounds are produced to make human sick, and the severity is individual dependent. In order to reduce the foodborne pathogen hazard, it is important to eliminate those bugs (factors) during food processing operations including packaging. The keys are to prevent the contamination and then, to kill all the harmful bugs in the food supply system. Since it is almost impossible to achieve this ideal goal, except the aseptic/retort process, the modeling technique may be used to predict the growth/survival potentials and to assess the risk.

Mathematical modeling is a scientific and systematic approach to study and describe the recurrent events or phenomena with successful application track for decades. When models are properly developed and validated, their applications save costs and time. For the microbial food safety concerns, models are developed based on the facts that most bacterial behaviors are reproducible, and can be quantified by characterizing the environmental factors affecting growth, survival, and inactivation. For food safety management, control and monitor, models may serve an effective and useful tool for risk assessment.

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MATERIALS AND METHODS

I. *Microbial Strains*

Experiments were carried out using a cocktail mixture of pathogens of 4 - 6 strains depending on the availability in Lab to cover a wide range of potential microbial hazard. The selected strains are typical from the food outbreaks and collected by the United States Department of Agriculture (USDA) or Food and Drug Administration (FDA). A loopful of each strain was transferred from a stock culture (stored at -80◦C) into 10 mL of brain heart infusion broth (BHI, Becton, Dickinson and Co., Sparks, Md., USA) and incubated at 37◦C for 6 h. A loopful of cell suspension of each strain was then separately transferred to 10 mL of BHI broth and incubated at 37◦C for 24 h. A cocktail was formed by mixing each individual strain which has been properly diluted to have similar microbial counts. A certain amount of the cocktail (e.g. 3 log CFU/g, CFU: colony forming unit) was then inoculated onto the selected food. Food samples were taken at certain time intervals for plate counts. Selective medium agar plates were used, e.g. *L. monocytogenes* – MOX, *Salmonella* – Rappaport, and *E. coli* O157 : H7 – CT-SMAC.

II. *Temperature and Other Parameters*

Microbial growth is largely affected by temperature and other physical parameters. Most food items, except the aseptically processed ones, may subject to spoilage and cross-contaminations. For frozen and refrigerated foods, abused temperature around 6 - 10ºC were selected for *E. coli* O157 : H7 and *Salmonella* and 2 - 10ºC for *L. monocytogenes*. The pH and moisture content at 7.0 and > 90%, respectively, were applied to these three common pathogens. Modified Atmosphere Packaging (MAP) was applied at N_2/CO_2 ratio of 50/50% vs. no MAP (ambient). Two antimicrobial agents, i.e. chitosan and allyl isothiocyanate (AIT), were investigated to examine their functions in film and MAP, respectively.

III. *Mathematical Model*

There are several simplified growth models (primary) available in the literature which may be used for data/curve fitting when a completed data set with lag time, exponential grow phase and maximum growth population was collected. Those models were derived from the observation data with first-order kinetic reaction theory to achieve the empirical models, e.g. the Gompertz model[4]; Baranyi and Roberts model[5]. Those models show microbial growth as a function of time at constant temperature. If other parameters (e.g. pH, water activity, etc.) are included in the growth models, the secondary models are needed. Secondary models may include the Square-Root-Type (Ratkowsky *et al.*)[6], polynomial-type and other models which can be developed by using experimental designs and regression procedures (v9.1, SAS). Factorial design and linear or non-linear regression were used in this report. Microbial surface transfer models during meat slicing were developed by the non-linear regression procedures.

IV. *Microscopic Instrumentation for Microbe Testing*

In order to observe the dead/live cells of microbes, it is necessary to use high resolution microscopy instrument. Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) may provide the details in cell structure to distinguish the dead/live cells under different stress. For Confocal Laser Scanning Microscope (CLSM) application, 5 μL aliquots of stain/dye (Live/Dead BacLight Bacterial Viability Kits – Invitrogen, Carlsbad, Calif., USA) was added, mixed with the microbial sample, and incubated for 15 min to identify the dead/live cells by the stained red/green color, respectively.

V. *"Smart" Microwave Oven*

A household microwave oven with an inverter was modified to include the infrared (IR) temperature sensor, feedback control device and software. The 'smart' microwave oven can monitor the food surface temperatures and regulate the inverter to deliver microwave power as required[7] which further improve cooking uniformity to achieve the thermal killing effect (lethality) in the food domain and to maintain food texture and qualities.

RESULTS AND DISCUSSION

I. *Contamination and Inactivation during Processing*

An example of microbial cross-contamination is the surface transfer of pathogens during slicing of ready-to-eat (RTE) meats. *L. monocytogenes, E. coli* O157 : H7 and *Salmonella* may be transferred from one surface to another contact surface with different potentials. The transfer potential also depends on other factors, e.g. foods, process parameters, etc. There is a 2 - 3 log CFU/slice gap between the inoculated (on blade surface) and transferred counts at the initial slicing stage. The discrepancy of cell counts could be due to the surface sheer stress impact. Figure 1 shows the live cells (green on blade surface before slicing) and dead cells (red on blade surface after 10 slices of agar slicing) under CLSM. Agar was used to make the CLSM images possible since meat contains proteins and phosphates which cause interference with the CLSM observation. The rpm of blade in the range between 100 and 300 was found not significant impact on cell dead. Figure 1 also demonstrates that the cells can be trapped into the rough blade surface. If cells remained alive, cross-contamination may occur anytime during

slicing to cause food safety risk.

The surface transfer models[8,9] are all empirical based on curve fitting to attain the simple and easy-to-use equations. *Salmonella* transfer models are shown as the following:

Transfer model for direct blade inoculation case:

$$Y = (0.301 \cdot n^{1.446}) \cdot X^{(-0.051n+0.061)} \quad (1)$$

For the contaminated meat (e.g. ham) to a clean blade to another clean meat (e.g. ham) case:

$$Y = 1.119 \cdot n^{0.713} \cdot X^{(-0.151)} \quad (2)$$

Where Y is the microbial counts in log CFU/sliced meat or log CFU/g; n: total initial cell counts of surface inoculation in log CFU; X: sliced meat index, e.g. 1st, 5th, and etc. If meat slicing rate is m per min, X can be replaced by m·t (t: time in min). However, X in Equation (1) and (2) is an integral to be satisfied by selecting the process time. Figure 2 shows the surface transfer pattern of meat slicing at n = 5.0 of three pathogens. Without the models, the surface transfer prediction may become challenge especially at the low microbial contamination level.

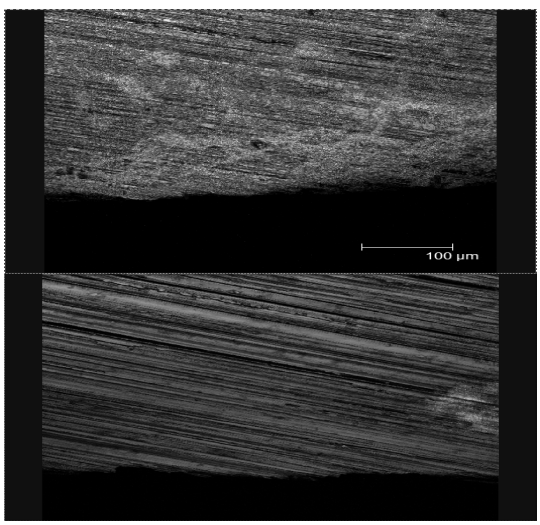

Figure 1. CLSM images of *Listeria monocytogenes* survival after surface stress impact due to slicing operation (12" round blade @ 300 rpm). Alive (green, before slicing) and dead (red, after slicing) on blade surface.

II. *Effects of Antimicrobial Agents and MAP on Microbial Survival*

There are many reported food-grade chemicals or ingredients which may be used to reduce or inhibit the microbial growth including chitosan[10] and natural essential oils. The effects of modified atmosphere packaging (MAP) and antimicrobial agent on fish fillet (results not yet published) indicated that using a proper combination of allyl isothiocyanate (AIT) and modified atmosphere (MA) may achieve the shelf life extension. Current study showed that lowering storage temperature as well as increasing AIT concentration (up to 36 ppm) can extend lag phase (LP) and reduce the growth rate (GR) of *Pseudomonas aeruginosa*. In addition, AIT alone reduced the *P. aeruginosa* counts within the first several hours. Results also showed that application of MA had a similar antimicrobial effect as lowering temperature. The synergy effect of AIT and MA combination extended the shelf life of fresh catfish fillet from 4 days (control) to 13 days at 8°C.

The shelf life is largely associated with the lag phase of the key microbe(s) (either spoilage or foodborne pathogens) in foods. A study to collect the survival data of certain microbe, *P. aeruginosa,* in a specified food system can assist in establishing the LP and/or GR using the available primary growth models (e.g. Baranyi). Thereafter, the secondary models to estimate the LP and GR of *P. aeruginosa* with impact of antimicrobial and/or MAP under abuse temperature conditions will be developed using the linear regression procedures (SAS) to attain polynomial equations. The results may assist food industry to predict the shelf life of catfish products which may be further optimized with proper AIC concentration in a MA package.

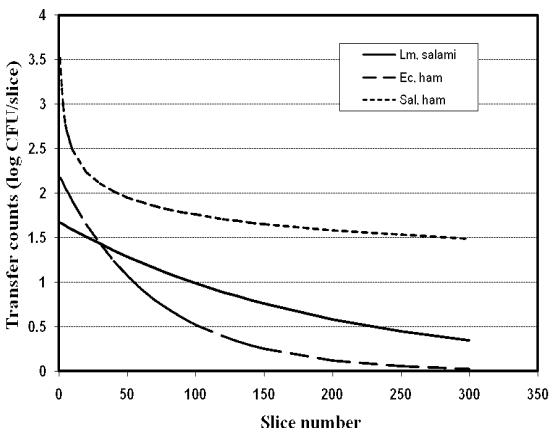

Figure 2. Transfer predictions using models with inoculation level at 5 log CFU for *L. monocytogenes* (Lm), *E. coli* O157 : H7 (Ec) and *Salmonella* (Sal) (surface-transfer from inoculated RTE meat to blade to clean RTE meat).

Chitosan has been reported an effective compound to inhibit the growth of foodborne pathogens including *L. monocytogenes, E. coli* O157 : H7 and *Salmonella* spp. The impact may be obvious in a liquid phase where the microbe has intimate contact with chitosan. However, in an in-mobilized structure the efficacy may be significantly reduced. In current study, an edible thin film was constructed by 90% of chitosan and 10% of other polysaccharide (e.g. cellulose), followed by inoculating microbes on film surface to examine the microbial survival. There is almost no impact on the microbe survival as shown in Figure 3, where the TEM image was taken after 24 h of contact. All cells remained intact on film surface. There is not damage of cells observed with longer contact time.

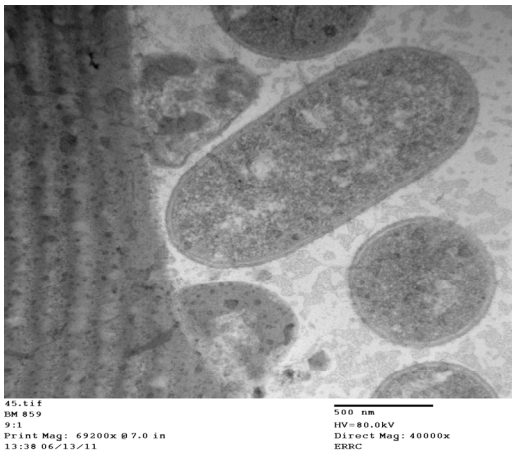

Figure 3. TEM image of *E. coli* O157:H7 on thin film (chitosan 90%: cellulose 10%, w/w, Left-hand side) surface after 24 h of contact.

III. *Microwave Heating Process – A 'New' Approach*

Microwave is well-known for its un-even heating in food cooking process resulting in microbial risk, especially, for the partially-cooked not ready-to-eat food items (NRTE). However, microwave oven may be better designed with IR temperature sensor and feedback control device to improve the cooking uniformity. The modified household oven may provide a reliable cooking tool to ensure consumer food products of foodborne pathogen free. Although there are some hurdles to be overcome, preliminary results showed that products, with best sensory quality, are attainable when combined with food ingredients (formulation) and packaging (microwave susceptor) technology. Fish fillet treated in phosphate solution may be well-cooked in microwave oven which has inverter and feedback control design. The products are juicy, tender and have a nice appearance which achieved by eliminating the 'bumping' during microwave cooking.

CONCLUSIONS

Many food factors are affected by processing and other preservation methods. Those factors include the nutrients, texture, appearance, and safety. Among them, safety is the most important concern for government, manufacturers and consumers. Model development and applications may enhance the food safety concerns with risk assessment. The Pathogen Modeling Program (PMP) and ComBase are two useful tools (a collection of models and data) to predict foodborne pathogen growth and inactivation in different foods. Using antimicrobial agents (chemicals and MAP) to enhance food safety may be valuable, but their effectiveness should be carefully evaluated in different food application systems. Microwave heating process may become a viable operation to achieve microbial food safety goal with build-in the proper control devices and design.

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