AN ABSTRACT OF THE THESIS OF

Nattaporn Chotyakul for the degree of Master of Science in Food Science and Technology, presented on December 1, 2009.
Title: Impact of Parameter Variability on the Food Process Engineering Calculations Required for Safety, Quality and Shelf-Life Estimations

Abstract approved: _____

J. Antonio Torres

Quantitative procedures were developed to determine the effect of variability in the model parameters required for the estimation of microbial shelf-life and thermal processing time. Monte Carlo simulations combined with these predictive models were implemented in Microsoft Excel^{TM}. In the first study, predictive models were used for shelf-life predictions based on the growth of *Lactobacillus sakei* in meat. The shelf-life values predicted when parameter variability was not considered were 3.6, 115.9, 4.1 and 144.8 h for cases 1 (T = 4°C), 2 (T = 4°C, a_w = 0.98), 3 (T = 4°C, CO_2 = 2,650 ppm), 4.1 (T = 4°C, a_w = 0.98, CO_2 = 2,650 ppm), respectively, whereas 3.9±1.7, 119.4±20.3, 4.6±1.4 and 160.4±0.3 h, respectively, were the values estimated considering parameter variability. The definition of a shelf life with 95% confidence that the product will not fail before the stated expiration date lead to a recommended microbial shelf-life of 2.5, 100, 3, and 110 h, respectively, When the reported standard deviation of all microbial model parameters describing the effect of the three factors in Case 4 (T =

4°C, a_w = 0.98, CO_2 = 2,650 ppm) was reduced by 10%, 50% and 90% without changing mean values, the recommended shelf-life time increased from 110 to 115, 125 and 130 h, respectively. This relatively small increase in the recommended shelf-life, i.e., an increase from 110 to 130 h after a 90% reduction in the variability of all model parameters, showed that reducing the standard deviation of microbial shelf-life time appeared difficult when assessing the effect of multiple factors.

In a second study, the estimation of a thermal process time at a constant reference temperature (T = 110°C) for the inactivation of *Clostridium botulinum* spores in commercially produced mushrooms, and based on the reported mean values for thermal inactivation time (D_T) and initial microbial load (N_o), yielded a recommended value of 5.96 min. Unique combinations of generated N_o^* and D_T^* datasets were used to obtain a distribution of the spore survival probability and the associated percentage of under processing. Next, the coefficient of variation (CV) for the percentage of under processing when using 2 to 500 generated datasets was calculated to determine that 100 was an acceptable minimum number of datasets to estimate 9.6 min as a recommended thermal processing time considering the experimental variability of the parameters D_T and N_o and yielding a 10^{-9} failure probability with a 95% confidence. The predictive procedures were used also to assess the impact of reducing the standard deviation (*SD*) of both N_o and $D_{110°C}$ by 10%, 50%, and 90% yielding 8.6, 7.8 and 6.4 min, respectively, as a recommended thermal process time at 110°C with 95% confidence.

In both applications of the procedures here developed, i.e., prediction or microbial shelf-life and design of a thermal process, the user reached a recommendation with 95% confidence using procedures that could be implemented in Microsoft Excel and based on concepts suitable for inclusion in an undergraduate food science program.

Impact of Parameter Variability on the Food Process Engineering Calculations
Required for Safety, Quality and Shelf-life Estimations

by
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I understand that my thesis will become a part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Nattaporn Chotyakul, Author

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CONTRIBUTION OF AUTHORS

Dr. J. Antonio Torres as major professor provided the laboratory facilities needed for this research and contributed to the research design, analysis of findings and the preparation of all manuscripts here included.

Dr. Gonzalo Velazquez provided specific expertise on the computational methods used in food process engineering, particularly those relying on Microsoft Excel, and contributed to the data analysis and preparation of the manuscripts here included.

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Introduction

All food processors face uncertainties when making process, formulation and storage decisions because all calculation parameters values have intrinsic and measurement variability. In this study, the focus is the development of calculation strategies considering this variability and thus producing calculation outputs to reach decisions with known confidence levels. A constraint in all procedures to be developed was that they must be possible to implement using only Microsoft Excel^{TM} spreadsheets and of an adequate complexity level for incorporation in undergraduate programs in food science and technology. The cases covered in this study focused on the development of such procedures for the estimation of the microbial shelf-life of refrigerated foods and the design of a heat food sterilization process for a low-acid food. Although thermal processing is the most commonly used food preservation technology, a review of textbooks presenting this subject showed that the impact of parameter variability on the selection of the time-temperature conditions eliminating pathogens and spoilage microorganisms has not been covered.

Shelf-life refers to the period of time during which a product still meets the quality and safety expectations of the consumer. During this time the product will be in storage, transportation, commercial distribution and then subjected to highly variable consumer handling conditions. The change in the quality and safety of food products during this time depends on numerous intrinsic and extrinsic factors. The intrinsic factors include ingredient composition and food formulation, water

activity, pH, and the load and type of microorganisms present in raw ingredients or coming from the food production or storage and distribution environment. Among many others, the extrinsic factors include food processing conditions, storage temperature, packaging, relative humidity, handling during retail operations and food preparation and consumption steps (Gudmundsson and Kristbergsson, 2008). Predictive models describe mathematically the effect of extrinsic and intrinsic parameters on microbial growth and are used to estimate the microbial shelf-life of foods (Ross, 1996; Ross, 1999; Peleg, 2006a; McDonald and Sun, 1999; Almonacid-Merino and Torres, 1991a; Almonacid-Merino and Torres, 1991b; Almonacid-Merino and others 1993b; Almonacid-Merino and Torres, 1993; Almonacid-Merino and Torres, 2009; Li and others 2007; Li and Torres, 1993a; Li and Torres, 1993b; Li and Torres, 1993c; McMeekin and others 1993a). The mathematical descriptions used in predictive microbiology quantify microbial growth and survival in foods. These mathematical models are classified as primary or secondary. Primary models describe growth, survival or inactivation of microorganisms under constant conditions. Secondary models describe how parameters from primary models behave under a range of conditions defined by intrinsic and extrinsic factors (Gudmundsson and Kristbergsson, 2008). Predictive microbiology was developed as a rapid and cost-effective tool to reduce the risk of reaching consumers with unsafe or spoiled food products. If a predicted model is used to estimate microbial shelf-life, the cost of bringing a food product to market

is reduced and the product development phase can be shortened.

Monte Carlo procedures were developed during World War II by physicists working on nuclear weapon projects in the Los Alamos National Laboratory (Metropolis, 1987). These procedures are used when it is not possible to compute an exact result using a deterministic algorithm. An example of a deterministic algorithm is a mathematical function because a function always produces the same output given a certain input (Figure 1). An application where you would want to use a Monte Carlo procedure is in the generation of the distribution frequency of all possible values for a given outcome. In this study, such procedures were developed to predict the probability that fresh meat may fail before its stated microbial shelf-life, and also to estimate quantitatively the risk of under processing when applying a certain thermal treatment for the preservation of mushrooms. These procedures required knowing the statistical distribution of all calculation parameters which was obtained from published data.

The use of predictive models for the assessment of microbial shelf-life depends not only on the initial microbial load, processing steps, post processing contamination risk, storage conditions and food properties but also on the statistical variability of the parameters used in the predictive model. In the evaluation of microbial shelf-life covered in this study, the procedures developed considered the reported variability in the predict microbiology models and in the initial microbial load but not in the value of other extrinsic and intrinsic food parameters (e.g.,

storage temperature). The effect of the latter had been covered in previous studies (Almonacid-Merino and Torres, 1991a; Almonacid-Merino and Torres, 1991b; Almonacid-Merino et al., 1993b; Almonacid-Merino and Torres, 1993; Almonacid-Merino and Torres, 2009). In the case of the thermal processing application, the procedures considered the reported variability in the initial microbial load and in the value of the decimal reduction time. Particularly important was considering that regulatory agencies require now specifying the confidence level that a pathogen risk specified by current food safety regulations (e.g., 5 decimal reductions in the pasteurization of juices) will be met.

Microsoft Excel was used to generate random numbers with a given probability distribution for all parameters involved to develop a procedure that generated a distribution of the predicted shelf-life values. This distribution was used to generate a recommended shelf-life such that products subjected to similar conditions will not fail before this stated time period with a 95% certainty. The predictive microbiology models used in the first study of this thesis considered the effect of temperature (T), water activity (a_w), and dissolved carbon dioxide concentration (CO_2) on the growth of *Lactobacillus sakei* in refrigerated meat products. In the case of the thermal processing application described in the second study of this thesis, the objective was to determine a processing time at a reference temperature (F_T value) to achieve the thermal inactivation of *Clostridium botulinum* spores in mushroom. The process safety objective was to ensure that only one in a

billion containers would fail. In both applications, i.e., prediction or microbial shelf-life and design of a thermal process, the user reached a recommendation with 95% confidence. In both studies, the impact of the probability distribution of the parameters in the thermal processing model calculations was assessed by additional Monte Carlo-type computer experiments. To assess the potential benefit of reducing parameter variability, the calculation procedures were repeated using the same mean values reported in the literature but with the reported standard deviation of the parameters involved reduced by 10, 50, and 90%. This information can be used to evaluate the cost of improving data acquisition or segregating products according to the value of critical parameters such as initial microbial load.

In summary, the objectives of the first study of this thesis research were:

(1) To estimate quantitatively the predicted shelf-life of a specific food product handled under a specific set of environmental conditions; and,

(2) To use a shelf-life frequency distribution instead of a single point value when recommending a microbial shelf-life meeting the quality expectation of the consumer with a specified degree of confidence.

In the second study, the objectives were:

(3) To generate a frequency distribution of the thermal processing time required to meet a specified microbial safety target; and,

(4) To use the thermal processing time distribution to recommend a process eliminating a microbial risk with a specified degree of confidence.

An objective common to both studies was:

(5) To assess the benefits of improving data acquisition or segregating products according to the value of critical parameters such as initial microbial load.
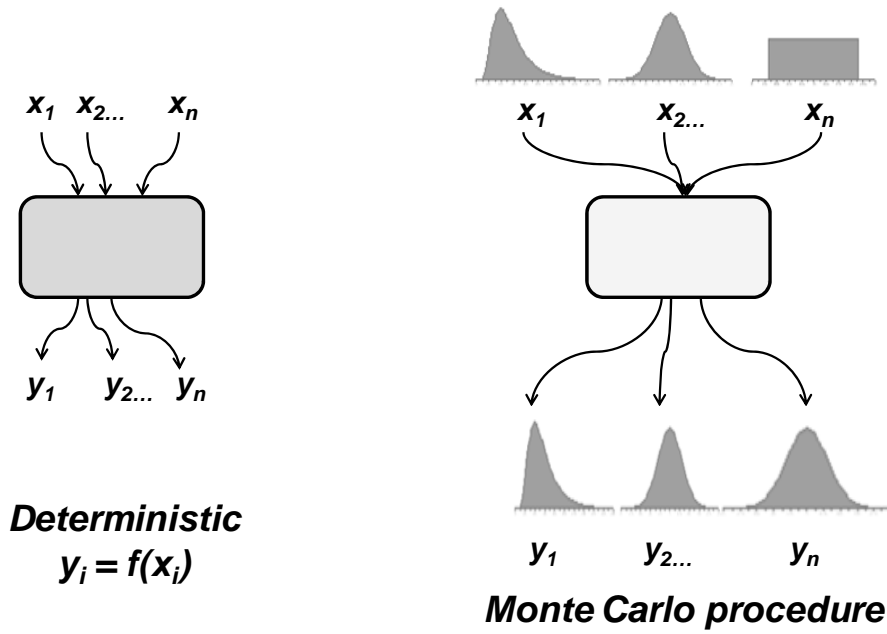
Figure 1. Schematic representation of the difference between Monte Carlo and conventional deterministic calculation procedures.

Literature Review

Food is essential for survival, growth, physical ability and good health. Therefore, its study has become a scientific discipline, "Food Science," and a critical endeavor for modern society. Although the evidence is still unclear, early reports indicate that humans were able to preserve a variety of foods in vinegar, brine, honey or pitch as recorded in the history of many societies. Other foods were salted or dried in the sun while cheese, wine, beer and other alcoholic beverages were obtained by microbial fermentations. Food preservation evolved as part of culinary arts from generation to generation but its development was slow until the later part of the eighteenth century when the improvement of existing and the development of new preservation methods accelerated greatly. The discovery of new technologies based on the application of science has resulted in great gains to human health by making available a varied, safe, nutritious and economical food supply (Vieira, 1996).

Modern Food Science covers the scientific principles to produce and maintain a high quality food supply in fresh and various preserved forms including canned and frozen products, refrigerated and shelf-stable ready-to-eat meals, beverages and snacks. A food scientist applies a wide range of scientific disciplines to make available to consumers a wholesome food supply including now improving the use of natural resources and minimizing waste generation during processing. The objective of food science education is to prepare professionals with the

knowledge required to process raw materials and other ingredients into foods. In addition to the application of chemistry and other science principles required for the design of food formulations, equally important are the applications of microbiology and engineering concepts for the design of the processing, packaging, storage and distribution conditions required for each food product. An additional constraint is the need to retain nutritional values and deliver foods that are a pleasure to eat (Anonymous, 2003).

The education of a food scientist can be defined as the one needed to apply science and engineering principles to study the physical, chemical and biochemical nature of foods and the principles of food processing (Potter and Hotchkiss, 1995). However, that is not enough and food scientist must also be able to communicate to consumers the role and benefits of each food ingredient and processing step in an understandable and precise manner. This is a difficult task which requires much thought, lots of efforts and excellent communication skills. However, if processor can achieve it, the rewards to society and even to the processor will be significant (Morton and Lenges, 1992). In his book entitled "Key Guide Food Science and Technology," Magnus Pyke defined the goal of food scientists and technologists as the capacity to process huge quantities of food required by an ever-growing population, making these foods acceptable to consumers (i.e., one that provides them with the type and quality of the foods they demand at all times), and to maintain and improve the nutritional value of the total food supply to protect the

health of the community (Green, 1985). A food scientist should not neglect the consumer perspective, i.e., establishing a constant dialogue between producers and consumer is important

Food science education has been considerably influenced by the recommendations developed by the Institute of Food Technologists (IFT), a professional society with membership from industry, academia and government organizations (Morton and Lenges, 1992). The recommendations regarding food science education are being followed by many academic institutions offering a food science program. Food scientists need to demonstrate educational outcomes in chemistry, microbiology, biochemistry, mathematics, physics, engineering, statistics, and in specialized courses such as food packaging. Current trends in food and drink consumption patterns requires the development of more elaborate and complex products with a higher use of technology to deliver closer to fresh-prepared products and delivering a higher retention of desirable functional components such as antioxidants (Morton and Lenges, 1992). The three dominant forces driving food demand are:

- Higher quality, or perceived higher quality, products (natural foods, light products such as low fat foods, high fiber products, products with fewer additives, environmentally friendly products, etc.).
- Products of greater convenience (ready-to-eat meals, microwavable products, chilled instead of frozen products, products to eat and drink on-the-go, etc.).

- Greater variety of products (sophisticated cuisine foods, ethnic foods, fresh foods, organic products, etc.).

The goal of food scientists is not only to develop and process foods but also to consider quality and safety criteria in the entire food chain from production, processing, distribution, storage and finally its consumption. The process is being influenced by the implementation of national (e.g., implementation of Good Manufacturing Practices, Hazard Analysis and Critical Control Points, etc.) and international (e.g., the World Trade Organization (WTO) Sanitary and Phytosanitary (SPS) Agreement) standards (Henson and Caswell, 1999). An important consideration is the determination of product shelf-life which requires the prediction of how quality and safety of foods changes with time, and the selection of processing conditions eliminating food spoilage and safety risks.

Shelf-life

Considerable research has been conducted on the quality evaluation of foods with an aim to determine shelf-life (Man and Jones, 2000). Shelf-life is the period of time before a food product reaches an unsatisfactory or unacceptable state under specific processing, packaging and storage conditions. In other words, it is the period of time during which it will retain an acceptable level of eating quality, from a safety, nutritional and sensory point of view. The four critical factors affecting this evaluation are composition, processing, packaging and storage

conditions (Steele, 2004). Shelf-life considerations should include also the currently increasing consumer demand for fresh, convenient, safe and superior quality foods. Food processor should approach the shelf-life determination methods with the necessary care to ensure that consumers will receive a high product quality with the added convenience of extended shelf-life (Steele, 2004). Also, food quality is a consumer-based perceptual construct which is relative to person, place and time (Cardello, 1995).

The Institute of Food Science & Technology in the United Kingdom states that during its shelf-life, foods stored as recommended by the producer should retain their desired sensory, chemical, physical, functional and microbiological characteristics (Man, 2002). Furthermore, their composition should comply with any label declaration of nutrition information. The estimation of shelf-life is today an important food development requirement to meet safety regulations and to deliver the consistency in quality required to meet consumer expectations. Ensuring that products do not exceed its shelf-life before consumption is an important responsibility for everyone in the food chain including the suppliers of ingredients, food processors, warehouse managers, supermarket operators, and even the final consumer. A consumer should know the length of time that a product can be kept at home before it can no longer be used. A retailer should know the length of time that a product can stay on store shelves while a manufacturer should know when a product is no longer marketable. Every food product should be described as having

a specific maximum microbiological, chemical and sensory shelf-life because every food will deteriorate at a different rate (Man, 2002). Although, shelf-life defined by the processor refers only to the unopened package, once a package is opened and its contents is not consumed in a single event, the rest should be stored under the conditions, particularly time and temperature, recommended by the manufacturer. In 1997, FDA determined that the labeling of potentially hazardous foods that need refrigeration should be more specific about the types of hazards present and the necessary storage conditions after the food is opened by consumers and issued labeling guidance to food manufacturers (Anonymous, 1997; Marth, 1998).

The evaluation of food spoilage required to estimate shelf-life can be classified into physical, chemical and microbiological changes (Man and Jones, 2000; Singh and Cadwallader, 2004; Perchonok, 2002):

- Physical changes are caused by mishandling of foods during harvesting, processing and distribution. Examples of common physical changes include freeze burn, textural and flavor changes of bakery products due to freezing, and growth of ice crystals due to temperature fluctuation during storage of frozen food products. This type of damage can be prevented by food formulation, careful handling, proper packaging and control of storage temperature.

- Chemical changes during food processing and storage depend on food composition, type of packaging and environmental factors. Chemical changes

causing food deterioration include enzymatic reactions, non-enzymatic browning, oxidative reactions and cross-linking of proteins.

- Microbiological changes depend on the type and level of microbial load and on food, packaging and environmental factors. Shelf-life may end because microbial growth causes undesirable sensory changes in flavor, appearance including color, odor, and texture, or because the food is unsafe for consumption.

A careful combination of microbiology, sensory analyses and chemistry tests is required to determine which microorganisms are the specific spoilage organisms of a particular food product (van Impe and others 2005; Gram and others 2002). Microbial shelf-life can be estimated directly by microbiological tests or by the use of predictive models based on the combination of microbiology, statistics and engineering science.

Microbial shelf-life can be based on the growth of a spoilage-causing organism reaching a level considered unacceptable to consumers. If the concern is the presence of a microbial pathogen, including those for which a zero-tolerance has been established, this level could represent the number of a microorganism that would be detected during a food safety inspection (Figure 2). In the case of a spoilage microorganism, the final load would be relatively high (log N_s) and would be reached after completion of the lag phase and subsequent exponential rate growth. In the case of a pathogen, the initial load would be extremely low,

particularly if there is a zero tolerance regulation in place, and that load would remain low for a long time thanks to food formulation factors controlling this pathogen. If the pathogen completes its lag phase, one can expect a very slow exponential growth rate but eventually it could reach a number that would be detectable by microbial test methods (log N_d) which could be used to define a microbial shelf-life.

Shelf life analysis depends on the examination of batches of samples until the shelf life becomes unacceptable. The use of appropriate and validated analytical methods and sampling plans are essential when performing shelf life analysis using standardized protocol (Donnelly and Mitchell, 2002). The rational for the microbial testing of foods falls into four general categories: (1) to determine safety; (2) to determine adherence to Good Manufacturing Practices (GMPs); (3) to determine the utility of a food or ingredient for a particular purpose; and, (4) to predict product stability. Food of acceptable and unacceptable quality can be distinguished by the application of microbiological criteria. One approach is a sampling plan which is a systematic way to assess the microbiological quality of food lots. A lot refers to a batch of products manufactured under the same conditions at the same time. For the product to be acceptable, the results from the microbial analysis must conform with limits appropriate to the product and the number of samples taken from the lot randomly. As the severity of the hazard being tested for increases, the stringency of the sampling plan will increase. For

example, spoilage can be regarded as more of a risk to the product than to the

consumer and so tests for indicators of shelf-life such as aerobic plate counts will

have the most indulgent sampling plans. When looking for pathogens, more

stringent sampling plans are appropriate and these become more demanding as the

severity of the illness that the pathogen causes increases. The plan stringency

should also take into account whether the food is to be consumed by a particularly

vulnerable population groups such as infants, the very old, or the very sick (Adams

and Moss, 2008c).

Food spoilage means that the original nutritional value, texture, of flavor of

the food has been damaged and is unsuitable to eat. Different conditions can

accelerate spoilage including inappropriate temperature and moisture control.

Product spoilage may be visually detected, e.g., loss of bright red color on meat

products or appearance of mold colonies on cheese. The rejection may be related

to the senses of taste and smell detecting levels of metabolites associated with

spoilage. Traditional microbiological methods to determine the extent of

deterioration are limited by the time required to obtain results and generally do not

give a response until a large numbers of cells are present. Formation of detectable

amounts of spoilage metabolites may require 10^7 cells/g or ml of the product, i.e.

the state of incipient spoilage is only a few further generations from overt spoilage

(McMeekin and Ross, 1996).

The application of sampling plans for microbiological criteria was

innovated by the International Commission on Microbiological Specifications for Foods (ICMSF) by the introduction of two- and three-class sampling plans (Dahms, 2004). In the case of the two-class sampling plan, the parameters are (Figure 3a): (1) n = number of samples drawn from the lot individually analyzed for the defect; (2) c = maximum allowable number of sample units yielding unsatisfactory results; and, (3) m = limit for defect acceptability. For example if $m = 10^5$cfu/g, samples resulting in counts of 10^3 and 10^6 cfu/g , would be considered acceptable and defective, respectively. The lot will be accepted if the number of defective samples is less than c. A lower c value will reduce the consumer's risk of receiving a defective product but increase the producer's risk of rejecting a lot of acceptable quality. In the case of a zero-tolerance pathogen for a ready-to-eat product, m and c are set to 0 but the values can be different from 0 for foods that will receive a heat treatment before consumption. That is why the sampling plans Salmonella in raw and cooked shellfish are different (Table 1).

In the case of a three-class sampling plan (Figure 3b), the definition of a fourth parameter (M) allows the grouping of lots into three categories: acceptable, marginal, and rejected. To enhance food safety and improve food quality, more stringent microbiological limits can be set by reducing value of m and M. The

The International Commission on Microbiological Specifications for Foods has developed a set of recommendations to classify foods according to their risk level (Adams and Moss, 2008c; Man, 2002; Swanson, 2009):

1) No direct health hazard concern but the product can spoil. The recommended sampling plan is 3-class with $n = 5$ and $c = 1$.

2) Low concern of health hazard based on an indicator of good manufacturing practices (GMP) such as coliform and Enterobacteriaceae counts. Again, the recommended sampling plan is 3-class with $n = 5$ and $c = 1$ but the m and the M values will be lower than in the previous case.

3) Moderate hazard of health concern and there is a need to limit the spread of the pathogen such as in the case of *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens*. The recommendation plan is a 3-class plan with $n = 10$ and $c = 1$).

4) Serious hazard of health concern as in the case of Samonella and *Yersinia, enterocolitica*. To reduce this hazard concern during lot acceptance testing, the recommendation is a 2-class sampling plan with $n = 20$ and $c = 0$.

5) Severe hazard of health concern as in the case of life threatening or serious pathological consequences to the consumer. Examples of microorganisms in this group are *Clostridium botulinum* toxin and *Escherichia coli* O157:H7. In this case, the recommended sampling plan is a 2-class with $n = 60$ and $c = 0$.

Predictive microbiology

A predictive model describes mathematically the response of a microorganism to a given set of environmental conditions (van Impe et al., 2005;

McMeekin et al., 1993a). Predictive models can be used to evaluate the effect of processing, distribution and storage operations on the microbial safety and quality of foods. Mathematical models are used to estimate microbial risk as a function of one or more inputs. Particularly useful are those approaches that rely on the statistical description of experimental data (e.g., mean and standard deviation) to generate data with the same statistical distribution. In this case, the predictive models generate descriptions of the variability and uncertainty of the estimated shelf-life. The complexity of the model is primarily due to the inclusion of probability distributions that describe the variability and uncertainty in the many parameters required by the model.

Poschet and others (2003) defined uncertainty as the lack of perfect knowledge of a quantity which may be reduced by additional measurements and by improvements of the measurement method. On the contrary, variability refers to the true heterogeneity of population which is irreducible by additional measurements. However, in actual practice it is difficult to differentiate between uncertainty and variability, especially when both have the same order of importance (Nauta, 2000). A Monte Carlo analysis can be used to illustrate the impact of uncertainty and variability of the experimental procedures used to generate model parameters and thus on the estimates generated by the predictive model (Poschet et al., 2003).

A Monte Carlo analysis is a computational tool using random number generation techniques to work with models that involve probability distributions to

describe model parameters (Cassin and others 1998). It refers also to procedures

where quantities of interest are approximated by generating many random values

(Figure 1). A Monte Carlo experiment will repeat calculations many times and

every time the outcome will be slightly different (Schmidheiny, 2008). In a

conventional calculation model, the input parameters have a certain value and an

output from those input values is obtained using equations. This type of model is

deterministic because the model results will be the same no matter how many times

they are recalculated. A Monte Carlo approach can be combined with a

deterministic model by using sets of randomly generated values as model inputs to

generate hundreds to thousands of model outputs. Because the input data are based

on random number generated from probability distributions to simulate the process

of experimental sampling, the researcher should select input distributions that most

closely describe the model parameters. The data generated from the Monte Carlo

simulations can be represented as probability distributions or histograms and the

conclusion reported as confidence intervals (Wittwer, 2004).

Thermal processing

Thermal processing is defined as a temperature and time combination

required for the inactivation of undesirable microorganisms and enzymes while

inducing an acceptable level of chemical changes in foods. The term commercial

sterility refers to a thermal treatment inactivating microorganisms that cause illness,

and are capable of growing under non-refrigerated storage and distribution

conditions (Toledo, 2007). Thermal processing has focused on the inactivation in

low-acid foods of spores of *Clostridium botulinum*, a heat resistant organism of

high public health risk to consumers (Clark, 2002). During the 1990-2000 period in

the U.S., 160 foodborne botulism outbreaks affected 263 people with the highest

incidences in Alaska, Idaho, and Washington (Sobel and others 2004). However,

these cases reflected home canning errors due to lack of proper training.

Although alternative models are being developed, the thermal inactivation

of microorganisms is most commonly described as first-order reaction kinetics, i.e.,

microbial survival is a logarithmic function of time at constant temperature. The

heat resistance of microorganisms is expressed in terms of a decimal reduction time

at a reference temperature T (D_T value*) reducing the number of microorganisms by

a tenfold (Hersom, 1975). The effect of temperature on D_T values is described by a

z value indicating the temperature increase required to decrease the death rate by a

tenfold (Toledo, 2007). Values of D_T and *z* are required to determine a food

sterilization process time at the reference temperature T (F_T value) and reducing the

initial bacterial spore load (*N*) to an acceptable final level (N_o). F_T values used

commercially are most often based on a bacterial spore causing product spoilage

and having a much higher heat resistant than spores of *Clostridium botulinum*

(Hersom, 1975; Toledo, 2007; Adams and Moss, 2008b; Stumbo and others 1975).

Conclusions

One of the most difficult decisions faced by a food processor is the need to respond to the modern consumer desire to have an estimate of the shelf-life, particularly when the product is as unstable as most refrigerated foods are. These estimations are expensive to obtain by microbiology methods and subject to large uncertainty when selecting storage testing conditions representing the actual commercialization of the product. Previous studies have covered the effect of water activity, packaging material, and particularly storage temperature which can rarely be assumed to be constant (Almonacid-Merino and Torres, 1991a; Almonacid-Merino and Torres, 1991b; Almonacid-Merino et al., 1993b; Almonacid-Merino and Torres, 1993; Almonacid-Merino and Torres, 2009; Li and Torres, 1993a; Li and Torres, 1993b; Li and Torres, 1993c; Torres and others 1994).

Predictive microbiology models are used in undergraduate food science program to expose students to the effect on microbial shelf-life of food properties (e.g., pH and a_w) and storage conditions (e.g., temperature). As described in this thesis document, Monte Carlo procedures can be used to estimate a microbial shelf-life with a known degree of confidence by considering the variability of all model parameters.

Thermal processing has remained the foundation of the processed foods industry, allowing the production of billions of shelf-stable containers of fruits, vegetables, soups, beverages and meats every year (Clark, 2002). Therefore,

teaching of food thermal processing should inform students about new

development in the mathematical description of microbial inactivation (e.g., Peleg,

2006b) and in predictive models strategy for the thermal processing of food

including considerations of the variability in model parameters using the Monte

Carlo procedures described in this thesis document.

Table 1
Examples of 2- and 3-class sampling plans for frozen crustaceans

Product	Organisms	Plan class	n	m	M	c
Raw	APC	3	5	10^6	10^7	3
	E.coli	3	5	11	500	3
	S.aureus	3	5	10^3	10^4	2
	Salmonella	2	5	0	-	0
	V.parahaemolyticus	3	5	10^2	10^3	1
Cooked	APC	3	5	5×10^5	10^7	2
	E.coli	3	5	11	500	2
	S.aureus	2	5	10^3	-	0
	Salmonella	2	10	0	-	0
	V.parahaemolyticus	3	5	10^2	10^3	1

Source: Adams and Moss (2008a)

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Figure 2. Definition of microbial shelf-life. (a) Microbial spoilage becomes unacceptable after completion of a lag time λ and exponential growth from an initial load to a maximum microbial load log N_s. (b) Microbial safety becomes unacceptable after completion of a long lag time λ and exponential growth from a very low initial load to a maximum microbial load log N_d a level that would be detected by the microbial sampling plan in place.

Figure 3. Microbial sampling plans. (a) 2-class sampling plans are most strict and the *m* value selected depends on the food safety objective defined for the product. Further sampling parameters are the number of samples *n* taken randomly from a production lot and the maximum number of samples *c* that can exceed the *m* value. (b) 3-class sampling plans are less strict as they define marginal lots, i.e., lots that have at most *c* samples that exceed the microbial load level *m* but are under the unacceptable load M. If one sample exceeds the value M, the lot is rejected.

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Effect of predictive model parameter variability on refrigerated microbial shelf-life

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Short version of title: Refrigerated microbial shelf-life …

Abstract

The assessment of product shelf-life should consider the contribution of the variable in all predictive model parameters. This study described the microbial growth of *Lactobacillus sakei* in meat products using Ratkowsky-type models. The predicted shelf-life without considering The shelf-life values predicted when parameter variability was not considered were 3.6, 115.9, 4.1 and 144.8 h for cases 1 (T = 4°C), 2 (T = 4°C, a_w = 0.98), 3 (T = 4°C, CO_2 = 2,650 ppm), 4.1 (T = 4°C, a_w = 0.98, CO_2 = 2,650 ppm), respectively, whereas 3.9±1.7, 119.4±20.3, 4.6±1.4 and 160.4±0.3 h, respectively, were the values estimated considering parameter variability. The definition of a shelf life with 95% confidence that the product will not fail before the stated expiration date lead to a recommended microbial shelf-life of 2.5, 100, 3 and 110 h, respectively, When the reported standard deviation of all microbial model parameters describing the effect of the three factors in Case 4 (T = 4°C, a_w = 0.98, CO_2 = 2,650 ppm) was reduced by 10%, 50% and 90% without changing mean values, the recommended shelf-life time increased from 110 to 115, 125 and 130 h, respectively. This relatively small increase in the recommended shelf-life, i.e., an increase from 110 to 130 h after a 90% reduction in the variability of <u>all</u> model parameters, showed that reducing the standard deviation of microbial shelf-life time appeared difficult when assessing the effect of multiple factors.

KEYWORDS: Monte Carlo, experimental variability, microbial shelf-life

Introduction

Shelf-life is the period of time during which a food product can be kept under a specified condition and still meet the quality expectations of the consumer group target. Food shelf-life is affected by numerous extrinsic parameters describing processing and storage conditions, and by intrinsic properties such as composition, pH and water activity. Predictive models describing mathematically the effect of these extrinsic and intrinsic parameters on microbial growth are now available to estimate the microbial shelf-life of foods (Ross, 1996; Ross, 1999; Peleg, 2006a; McDonald and Sun, 1999; McMeekin et al., 1993a). These models allow estimations of lag time, generation time and exponential growth rate (McDonald and Sun, 1999). Koutsoumanis and Nychas (2000) used predictive modeling to determine the shelf life of various fish products as affected by temperature and water activity. Fernandez and others (1997) developed a mathematical model for the combined effects of temperature (4-20°C), CO_2 (0-100% v/v, balance N_2), pH (4.5-7.0) and NaCl concentration (0.5-8.0% w/v) on the growth of *Listeria monocytogenes*. The effects of 115 combinations of these factors were examined. Using the model proposed by Baranyi and Roberts (1995), predictions for doubling time, specific growth rate and time to a 1000-fold increase could be calculated for any combination of conditions within the experimental matrix. The model was successfully validated by comparing predicted growth values with published data for *L. monocytogenes* growth in a variety of foods

packaged under modified atmosphere. The effects of storage temperature and the permeability of the packaging film on microbial growth in refrigerated beef were mathematically modeled by Giannuzzi and others (1998).

The use of predictive models for the assessment of microbial shelf-life depends not only on initial microbial load, processing steps, post-processing contamination risks, storage conditions, and food properties but also on the statistical variability of the parameters describing them. Therefore variability considerations should be included in all predictive model calculations (e.g., Almonacid-Merino and Torres, 2009; Chotyakul and others 2009).

Recent food process engineering education reports have covered learning styles (Palou, 2006), comparison of knowledge gains and attitudes using computer-based and face-to-face personal hygiene training methods (Fenton and others 2006), construction of Internet-assisted real-time experiments (Singh and Circelli, 2005), development and use of a web site with multimedia contents to assist unit operations courses (Tapia and others 2005), and web-based calculation tools to assist food engineering courses (Morales-Blancas and others 2003; Morales-Blancas and Torres, 2004). However, the inclusion of parameter variability considerations was not found in food process engineering education reports. Variability considerations are particularly important in the case of predictive microbiology models due to the large number of parameters involved.

The high relative moisture content of fresh meats and the frequency of

temperature abuse shorten their shelf-life (Almonacid-Merino et al., 1993b; Almonacid-Merino and Torres, 1993; Almonacid-Merino and Torres, 2009; Torres, 1989). In this study, product shelf life was calculated using data published for natural contamination levels in meats (Martín and others 2006) and dissolved carbon dioxide level when modified atmosphere packaging (MAP) technology is used (Jakobsen and Bertelsen, 2002; Jakobsen and Bertelsen, 2004). Predictive model calculations were applied to examine the effect of temperature (T), water activity (a_w) and dissolved carbon dioxide concentration (CO_2) on the growth of *Lactobacillus sake* (*L. sake*), now renamed *Lactobacillus sakei* (*L. sakei*) by the International Code of Nomenclature of Bacteria (Trüper and de'Clari, 1997; Champomier-Vergès and others 2001). These conditions can be used to predict shelf life and microbial safety and to identify critical points in product manufacturing and distribution (Zwietering and others 1990; Zwietering and others 1991).

     Lactobacillus sakei is a gram-positive anaerobic bacterium commonly found in fresh meat and fish products. Some strains of this lactic acid bacteria (LAB) group are used as a microbial starter in fermented foods such as sausages, producing lactic acid to inhibit spoilage and pathogenic bacteria (Borch and others 1996). However, other strains produce exopolysaccharides yielding a slimy appearance that cause meat spoilage (Champomier-Vergès et al., 2001).

     To achieve longer microbial shelf-life time and to improve the safety of

fresh meats, carbon dioxide is used as modified atmosphere packaging (MAP) gas (Devlieghere and others 1998; McMillin, 2008; Devlieghere and Debevere, 2000). Carbon dioxide dissolved in the meat lowers its pH and inhibits oxidation processes. Both factors decrease the rate of chemical and biochemical deterioration reactions and thus product shelf-life is extended (Jakobsen and Bertelsen, 2002; Aymerich and others 2006).

The purpose of using predictive models is to estimate quantitatively the expected shelf-life of a specific food product stored and distributed under a particular set of environmental conditions (Soboleva and others 2000). However, food processors and distributors face uncertainties when they use these predictive models because of the statistical variability of the many parameters involved. Variability sources include processing temperature control, characterization of the raw material source including initial microbial load, and the determination of the intrinsic properties of foods. Variability in these parameters cannot be avoided and must be considered in shelf-life estimations. In addition, one must consider also the variability of the parameters in the predictive model equation used which is the objective of this study.

Modified Ratkowsky predictive microbiology models (McMeekin and others 2002; McMeekin and others 1993b) and Monte Carlo simulation methods (Floschet and others 2003) were combined to assess the impact on product shelf-life of the variability of the model parameters involved. The goal was to obtain

frequency distributions of shelf-life instead of single point values based on mean values for the parameters involved. To assess the impact of parameter variability, meat shelf-life was estimated using the same mean values but with a 0, 10, 50 and 90% reduction of the reported standard deviation of the parameters involved in the predictive model. The values obtained were compared with the shelf-life determined on the basis of the reported mean parameter values for all parameters. All these food process engineering and statistical methods were combined into MS Excel spreadsheets suitable for an undergraduate food process engineering course.

Material and methods

Predictive model

In this study, storage temperature T was assumed constant at 4ºC. Water activity (a_w) for meat has been reported to be no less than 0.98 (Rödel, 2001). This value was assumed constant too and can be measured with a small error of ±0.003 (Anonymous, 2006). The concentration of dissolved carbon dioxide (CO_2) used was the value reported by Jakobsen and Bertelsen (2002; 2004) for chopped pork (2650 ppm).

The primary model used in this study to describe microbial growth mathematically was a first-order growth model (Eq. 1) quantifying the number of microorganisms N as a function of time t. After a certain lag phase (λ) elapses during which cell numbers remain relatively constant, the number of

microorganisms increase rapidly at an exponential growth rate (μ_{max}) (Zwietering et al., 1990; Adams and Moss, 2008b; Brocklehurst, 2004; McKellar and Lu, 2004; van Impe et al., 2005). This approach defined a two-phase microbial growth model to estimate shelf-life (Figure 4) (Buchanan and others 1997; Zwietering et al., 1991; Zwietering et al., 1990). Shelf-life was defined as the time t_s required to reach a certain microbial load N_s at a growth rate μ_{max} from an initial contamination level N_o plus the lag phase time λ (Eqs 2-3).

$$\frac{dN}{dt} = \mu_{max} N \qquad \text{after t} = \lambda \qquad (1)$$

$$\frac{\log N_s - \log N_o}{\mu_{max}} = t_s \qquad (2)$$

$$Shelf\ life = \lambda + t_s \qquad (3)$$

Shelf-life calculations

Secondary model expressions for the lag phase (λ) and the exponential growth rate (μ_{max}) were proposed by Ratkowsky et al. (1982) who used a simple square root model considering only the effect of temperature. The secondary predictive models used in this study were modifications of these equations (Table 1, Koutsoumanis and Nychas, 2000) to estimate microbial shelf-life as a function of temperature only (Case 1); temperature and water activity (Case 2); dissolved carbon dioxide concentration and temperature (Case 3); and, temperature, water

activity and dissolved carbon dioxide concentration (Case 4.1). The initial

Lactobacillus sakei contamination level (log N_o) used to generate 300 static random

microbial load numbers with a lognormal distribution was the value reported for

naturally contaminated meat, 3.40 ± 0.34 log CFU/g (Martín et al., 2006). The

mean and standard deviation values for each parameter in the predictive

microbiology models (Table 3) were used to generate 300 static random numbers

with a normal distribution. With no repetition, each of the 300 generated initial load

and parameter values were used to obtain 300 values for λ and μ_{max}. Finally, a

shelf-value was calculated for each λ and μ_{max} using *log N_s = 6* as the end point for

L. sakei based on the recommended range of 10^6-10^7 CFU/g for lactic acid bacteria

as the value causing microbial spoilage of meat (Gram et al., 2002). The 300 shelf-

life values obtained for each case analyzed in this study by this Monte Carlo

simulation procedure generated histograms describing the probability distribution

of the expected shelf-life for meat. A recommended shelf-life was defined as a time

equal or shorter than 95% of the values in this histogram.

Effect of reducing model parameter variability

The Monte Carlo simulations previously described, and using the same

mean values, were repeated but assuming a 10%, 50% and 90% reduction in the

predictive microbiology model parameters (Cases 4.2-4.6) and also for the initial

microbial load (Case 4.7).

Results and discussion

Microbial shelf-life estimation

The first step in the evaluation of microbial shelf-life is to determine the initial microbial load on the product under consideration. In this study, the value used was the *L. sakei* load reported for meat by Martin and others (2006). The reported logmean and standard deviation values were used to generate random microbial load numbers following a lognormal distribution. The comparison of the reported logmean and standard deviation values with the ones calculated for the generated lognormal distribution (n = 300) showed an excellent agreement (Table 4) confirming the assumption of lognormal distribution for the microbial load. Similar good agreement was found for all other generated model parameters (data not reported).

The estimation of microbial shelf-life based on the four predictive microbiology models (Cases 1-4.1, Table 2) illustrated the importance of considering the variability of the parameters required (Figure 5). The shift to longer shelf-life when including a_w and CO_2 in the microbial model was expected. These factors extended the lag phase and reduced the growth rate. However, the variability of the predicted shelf-life values was not possible to predict a priori. The behavior observed highlights the advantages of the Monte Carlo procedures used in this study. Finally, the short shelf-life predicted by Case 1 suggests that it is not reasonable to ignore the effect of the meat a_w. In this study, the predictive model

calculations were based on the lower value in the 0.99-0.98 range reported by Rödel (2001).

The variability of the predicted shelf-life values was minor when the model considered only one factor (Case 1: $T = 4^oC$, Figure 5a) but increased significantly when the model included two factors (Case 2: $T = 4^oC$ and $a_w = 0.98$, Figure 5b; Case 3: $T = 4^oC$ and $CO_2 = 2650$ ppm, Figure 5c) or three factors (Case 4.1: $T = 4^oC$, $a_w = 0.98$, and $CO_2 = 2650$ ppm, Figure 5d). Significant differences can also be observed between the mean shelf-life values estimated when considering only the mean values of the model parameters and those obtained considering their variability (3.6 versus 3.9 ± 1.7 h, 115.9 versus 119.3 ± 17.4 h, 4.1 versus 4.6 ± 1.4 h, and 144.8 versus 160.4 ± 40.3 h for Cases 1-4.1 respectively, Table 5).

Considering the large variability observed in the estimation of microbial shelf-life, particularly when more than one preservation factor is considered, the recommendation to food processors would be to use a shelf-life value equal or shorter than 95% of the predicted values (n = 300 used in this study). Following this recommendation, the estimated shelf-life to use in product distribution would be 2.5, 100, 3 and 110 h for Cases 1-4.1, respectively (Table 5).

Effect of 10%, 50% and 90% reduction in the standard deviation of the predictive microbiology model parameters

The influence on the predicted shelf-life of the experimental variability of

the parameters in the Case 4 model was studied systematically. The reported

standard deviation of one or more parameters was reduced by 10%, 50% and 90%

(Case 4.2-4.6, Table 2) without changing their mean values to determine if the

variability of the predicted microbial shelf-life could be reduced significantly by

improving the experimental determination of these parameters or segregating

products according to initial microbial load (Figures 6 and 7).

The estimated shelf-life based on the original parameter variability was

160.4 ± 40.3 h. Reducing the variability of the parameter $a_{w\,min}$ (Figure 6a) by 10%,

50% and 90% resulted in estimated shelf-life time of 160.4 ± 40.0 h, 160.4 ± 40.2 h

and 160.3 ± 40.0 h, respectively (Case 4.2, Table 5) while reducing the variability

in T_{min} (Figure 6b) the equivalent vales were 158.3 ± 38.8 h, 158.6 ± 38.9 h and

157.6 ± 37.4 h (Case 4.3, Table 5). In these two cases, the effect was negligible

(Case 4.3) or minor (Case 4.3). The effect of reducing the variability in the

parameters b_4 and b_5 by 10%, 50% and 90% (Figure 6c) reduced more significantly

the variability of the predicted shelf-life yielding 160.7 ± 39.0 h, 157.3 ± 32.4 h and

154.6 ± 29.7 h (Case 4.4, Table 5), respectively. A similar effect was observed when

reducing the variability of the $CO_{2\,max}$ parameter (Figure 6d) by 10%, 50% and

90% yielding as estimated shelf-life values 159.4 ± 38.3 h, 155.4 ± 29.4 h and

153.6 ± 27.3 h (Case 4.5, Table 5), respectively. The impact of reducing at the same

time the variability of b_4 and b_5, $a_{w\,min}$ T_{min}, $CO_{2\,max}$ by 10, 50 and 90% yielded

shelf-life of 157.1 ± 33.6 h, 149.2 ± 17.9 h and 146.1 ± 10.5 h (Case 4.6, Table 5),

respectively, i.e., a significantly larger reduction in the variability of the predicted shelf-life.

Effect of the variability of the model parameters on the recommended microbial shelf-life

The motivation to reduce the variability of the shelf-life estimated using predicted microbiology models is to yield a longer value without increasing the risk of offering the consumer a product that has suffered microbial spoilage. Using the recommendation of a 95% confidence (n = 300) that such event will not occur, no increases could be recommended above the 110 h value estimated for Case 4.1 (i.e., original SD values for all parameters) when the variability of the parameters $a_{w\,min}$ and T_{min} was reduced by 10, 50, and 90% (Table 5, Case 4.2 and 4.3). An increase to 115 and 120 h was estimated when the variability of the parameters b_4, b_5 or $CO_{2\,max}$ was reduced by 50 and 90%, respectively, but not when the reduction was only 10 % (Table 5, Cases 4.4 and 4.5). A larger effect was observed when the variability of all parameters was reduced by 10 (Figure 7a), 50 (Figure 7b) and 90% (Figure 7c). In this case, the recommended shelf life would be 115, 125 and 130 h (Table 5, Case 4.6) when the variability of all parameters was reduced by 10, 50 and 90%, respectively.

The effect of the variability of the initial microbial load on shelf-life

The low variability in the predicted shelf-life for Case 1, i.e., temperature as the only one storage factor affecting the lag phase and exponential growth rate of *L. sakei*, suggested that the larger variability of Case 2-4.6 was not due to the variability in the initial microbial load. This was confirmed by examining the effect of reducing the variability of the microbial load by 10 (Figure 8a), 50 (Figure 8b) and 90% (Figure 8c) yielding a small change from 110 to 115 h in the recommended shelf-life only when the reduction was 90% (Table 5, Case 4.7).

Conclusions

Predictive microbiology has been developed as a rapid and cost-effective tool to reduce the risk of reaching consumers with unsafe or spoiled food products. These mathematical models allow the exploration of multiple factors including microbial load of raw materials, food formulation, processing steps, packaging strategies including modified atmosphere packaging (MAP), as well as the conditions found during storage, shipping and distribution. The laboratory experiments to test all these microbial stability factors would be complex and prohibitively expensive. Although the computer implementation of these models reduces this cost, their effectiveness depends on the determination of the values for several parameters under conditions at least similar to the application of interest. This study examined the impact of the variability of Ratkowsky-type model

parameters for lag phase (λ) and exponential growth rate (μ_{max}) used to estimate the microbial shelf-life of meat. Random number generations for lognormal and normal distributed parameters combined with Monte Carlo simulations were successfully implemented using Microsoft Excel to determine the probability distribution of shelf-life calculated using these predictive models. This allowed the determination of a procedure to estimate a recommended shelf-life considering the variability in microbial load and other model parameters. This is important since regulatory agencies have begun to require evidence that safety targets are met with a certain probability considering the variability of the calculation parameters. This requirement is typically set at 95% confidence interval (CI) or higher (Javis, 1989; Fernandez and others 1999; Rieu and others 2007; Smout and others 2000b).

The shelf-life values obtained considering the variability of the information required when using predictive microbiology models differed significantly from simpler calculations using only mean values for all parameters (Table 5). The strategy of implementing the generation of model parameter values following known statistical distributions and Monte Carlo simulations in the form of Excel spreadsheets should be presented in food process engineering courses covering estimations of microbial shelf-life. Excel implementation instructions can be obtained from the corresponding author.

Table 2
Predictive microbiology models

Case	λ (h)	Eq	μ_{max} (h^{-1})	Eq

a. Models used with original parameter values (McKellar and Lu, 2004; Devlieghere and others 1999)

$$1\ (T) \qquad \lambda\ =\ \frac{1}{b_1\,(T-T_{min})^2} \qquad (4) \qquad \mu_{max}\ =\ b_1\,(T-T_{min})^2 \qquad (5)$$

$$2\ (T,\ a_w) \qquad \lambda\ =\ \frac{1}{b_2\,(a_w-a_{w\,min})(T-T_{min})^2} \qquad (6) \qquad \mu_{max}\ =\ b_3\,(a_w-a_{w\,min})(T-T_{min})^2 \qquad (7)$$

$$3\ (T,\ CO_2) \qquad \lambda\ =\ \frac{1}{b_4\,(CO_{2\,max}-CO_2)(T-T_{min})^2} \qquad (8) \qquad \mu_{max}\ =\ b_5\,(CO_{2\,max}-CO_2)(T-T_{min})^2 \qquad (9)$$

b. Model used with reported and reduced standard deviation (SD) values for its parameters

$$(T,\ a_w,\ CO_2) \qquad \lambda\ =\ \frac{1}{b_4\,(a_w-a_{w\,min})(CO_{2\,max}-CO_2)(T-T_{min})^2} \qquad (10) \qquad \mu_{max}\ =\ b_5\,(a_w-a_{w\,min})(CO_{2\,max}-CO_2)(T-T_{min})^2 \qquad (11)$$

4.1   Mean and SD for all parameters as reported in the literature (Devlieghere et al., 1999)

4.2   Reported mean and 10%, 50%, 90% SD reduction of $a_{w\,min}$

4.3   Reported mean and 10%, 50%, 90% SD reduction of T_{min}

4.4   Reported mean and 10%, 50%, 90% SD reduction of $b_4,\ b_5$

4.5   Reported mean and 10%, 50%, 90% SD reduction of $CO_{2\,max}$

4.6   Reported mean and 10%, 50%, 90% SD reduction of all parameters

4.7   Reported mean and 10%, 50%, 90% SD reduction of the microbial load

Table 3
Predictive microbiology parameters describing the lag phase (λ) and maximum specific growth rate (μ_{max}) for *Lactobacillus sakei*

Eq.	Parameter	Mean $\pm$ standard deviation	Source
(4), (5)	b_1	0.0207 ± 0.0008	(McKellar and Lu, 2004)
	T_{min}	-2.93 ± 1.27	
(6)	b_2	0.012 ± 0.001276	(Devlieghere et al., 1999)
	$a_{w\,min}$	0.9469 ± 0.000867	
	T_{min}	-2.31 ± 0.308673	
(7)	b_3	0.0141 ± 0.001840	(Devlieghere et al., 1999)
	$a_{w\,min}$	0.9561 ± 0.001071	
	T_{min}	-8.1 ± 1.071429	
(8)	b_4	$9.3E\text{-}07 \pm 1.96E\text{-}07$	(Devlieghere et al., 1999)
	$CO_{2\,max}$	$1.4E04 \pm 2602.041$	
	T_{min}	-2.38 ± 0.290816	
(9)	b_5	$2.5E\text{-}06 \pm 3.57E\text{-}07$	(Devlieghere et al., 1999)
	$CO_{2\,max}$	$6.1E03 \pm 586.7347$	
	T_{min}	-9.0 ± 0.892857	
(10)	b_4	$9.3E\text{-}07 \pm 1.96E\text{-}07$	(Devlieghere et al., 1999)
	$a_{w\,min}$	0.9470 ± 0.000765	
	$CO_{2\,max}$	$1.4E04 \pm 2602.041$	
	T_{min}	-2.38 ± 0.290816	
(11)	b_5	$2.5E\text{-}06 \pm 3.57E\text{-}07$	(Devlieghere et al., 1999)
	$a_{w\,min}$	0.9560 ± 0.000816	
	$CO_{2\,max}$	$6.1E03 \pm 586.7347$	
	T_{min}	-9.0 ± 0.892857	

Other model parameter conditions: $T = 4$ ºC, $a_w = 0.98$, $CO_2 = 2650$ ppm, log $N_o = 3.40 \pm 0.34$ (Martín et al., 2006)

Table 4
Mean and standard deviation for *Lactobacillus sakei* (*log N$_o$*) in naturally contaminated meat[1]

Reported[1]	Calculated from random lognormal generated values (n = 300)			
	Case 1	Case 2	Case 3	Case 4 (all)
3.40 ± 0.34	3.39 ± 0.33	3.40 ± 0.34	3.38 ± 0.35	3.36 ± 0.34

[1](Martín et al., 2006)

Table 5
Predicted microbial meat shelf-life

Case	Mean-value calculations		Monte Carlo simulations Mean ± standard deviation (h) [Shelf life estimate, 95% confidence]		
1	3.6		3.9 ± 1.7 [2.5]		
2	115.9		119.3 ± 17.4 [100]		
3	4.1		4.6 ± 1.4 [3]		
4.1	144.8		160.4 ± 40.3 [110]		
		Reducing the predictive microbiology model parameters variability			
		SD reduction	10%	50%	90%
4.2		$a_{w\,min}$	160.4 ± 40.0 [110]	160.4 ± 40.2 [110]	160.3 ± 40.0 [110]
4.3		T_{min}	158.3 ± 38.8 [110]	158.6 ± 38.9 [110]	157.6 ± 37.4 [110]
4.4		b_4, b_5	160.7 ± 39.0 [110]	157.3 ± 32.4 [115]	154.6 ± 29.7 [120]
4.5		$CO_{2\,max}$	159.4 ± 38.3 [110]	155.4 ± 29.4 [115]	153.6 ± 27.3 [120]
4.6		all parameters	157.1 ± 33.6 [115]	149.2 ± 17.9 [125]	146.1 ± 10.5 [130]
		Reducing the initial microbial load variability			
		SD reduction	10%	50%	90%
4.7		Log N_o	158.4 ± 39.0 [110]	159.1 ± 38.8 [110]	159.2 ± 38.6 [115]

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Figure 4. Definition of shelf-life based on a two-phase microbial growth model, i.e. a lag phase (λ, h) followed by exponential growth rate (μ_{max}, h^{-1}) before reaching a maximum acceptable microbial load (log Ns).

Figure 5. Distribution of the predicted shelf-life for meat based on the growth of *Lactobacillus sakei* and reaching 10^6 cfu/g as the shelf-life endpoint. The predictive microbiology models considered one or more storage conditions. (a) Temperature (T, Case 1); (b) Temperature and water activity (T and a_w, Case 2); (c) Temperature and dissolved CO_2 (T and CO_2, Case 3); and, (d) All three factors (T, a_w and CO_2, Case 4.1). The values for the storage conditions used were $T = 4$ ºC, $a_w = 0.98$, and $CO_2 = 2650$ ppm. The shelf-life predicted by these models reflected the variability in the initial microbial load and the model parameters (see Table 3).

Figure 6. Effect of reducing by 10%, 50% and 90% the standard deviation (SD) value of the predictive microbiology model parameters (see Table 3, Case 4.2-4.5) used to estimate meat shelf-life based on the growth of *Lactobacillus sakei*. The model used considered storage temperature ($T = 4$ ºC), water activity ($a_w = 0.98$) and dissolved CO_2 ($CO_2 = 2650$ ppm). Variability in the shelf-life predicted reflected the variability in the initial microbial load and the model parameters (see Table 3). (a) Case 4.2: Reducing the SD for $a_{w\,min}$; (b) Case 4.3: Reducing the SD for T_{min}; (c) Case 4.4: Reducing the SD for b_4 and b_5; and, (d) Case 4.5: Reducing the SD for $CO_{2\,max}$.

Figure 7. Effect of reducing the standard deviation (SD) values of the predictive microbiology model parameters T_{min}, $a_{w\,min}$, b_4, b_5, and $CO_{2\,max}$ (see Table 3, Case 4.6) used to predict meat shelf-life based on the growth of *Lactobacillus sakei*. This model includes the storage conditions temperature (T = 4 ºC), water activity ($a_w = 0.98$) and dissolved CO_2 ($CO_2 = 2650$ ppm). Variability in the shelf-life predicted reflected the variability in the initial microbial load and the model parameters (see Table 3). (a) Reducing SD values by 10%; (b) Reducing SD values by 50%; and, (c) Reducing SD values by 90%.

Figure 8. Effect of reducing the standard deviation (SD) value of the initial microbial load (see Table 3, Case 4.7) used to predict meat shelf-life based on the growth of *Lactobacillus sakei*. This model includes the storage conditions temperature (T = 4 ºC), water activity ($a_w = 0.98$) and dissolved CO_2 ($CO_2 = 2650$ ppm). Variability in the shelf-life predicted reflected the variability in the initial microbial load and the model parameters (see Table 3). (a) Reducing SD value by 10%; (b) Reducing SD value by 50%; and, (d) Reducing SD value by 90%.
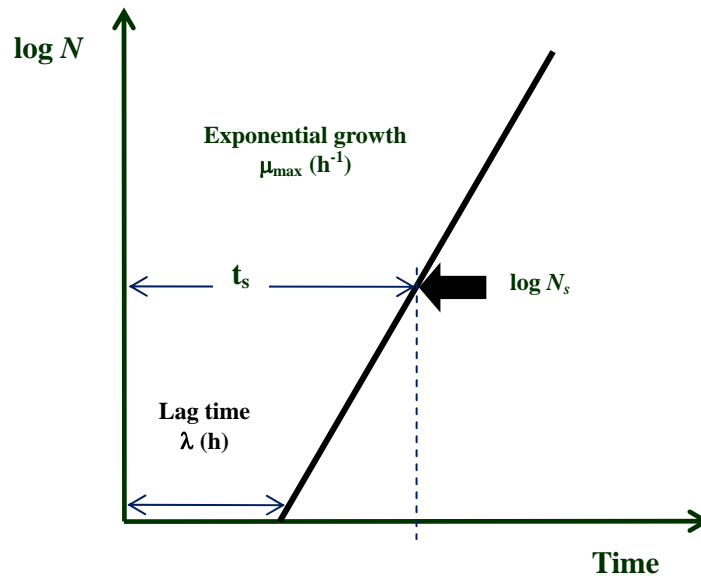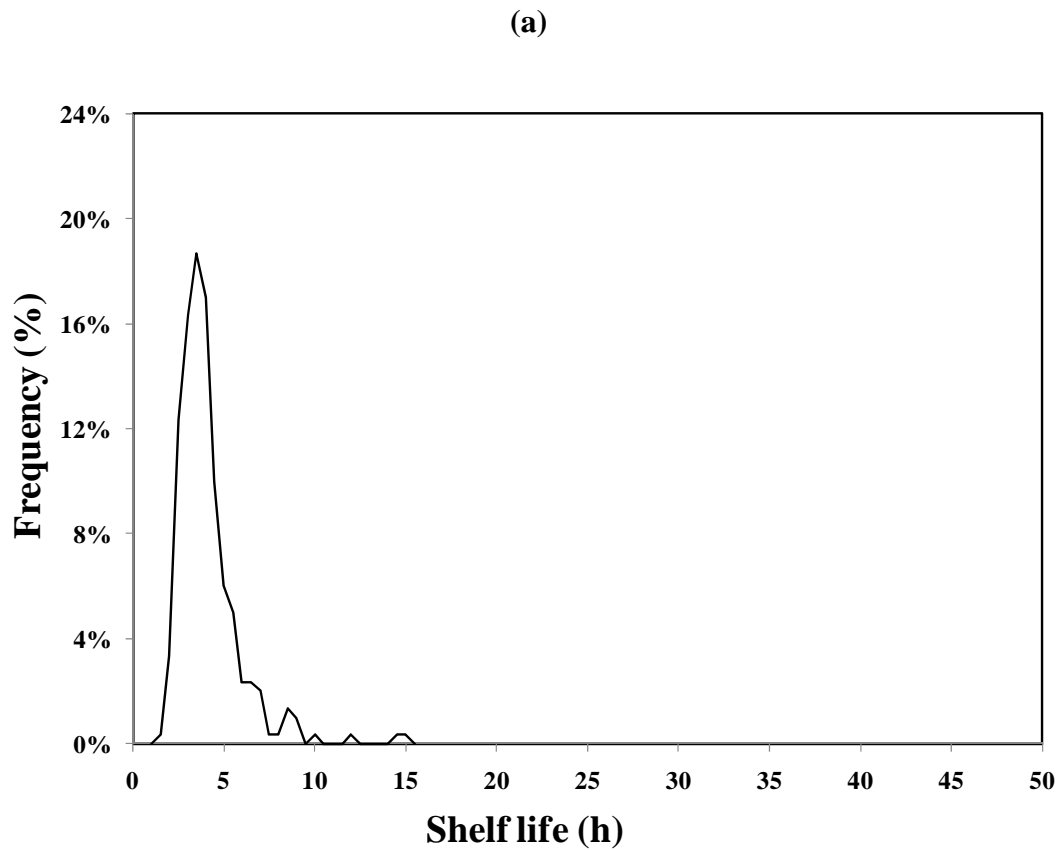
Figure 4. Definition of shelf-life based on a two-phase microbial growth model, i.e. a lag phase (λ, h) followed by exponential growth rate (μ_{max}, h^{-1}) before reaching a maximum acceptable microbial load (log N$_s$).

(a)

Figure 5. Distribution of the predicted shelf-life for meat based on the growth of *Lactobacillus sakei* and reaching 10^6 cfu/g as the shelf-life endpoint. The predictive microbiology models considered one or more storage conditions. (a) Temperature (*T*, Case 1); (b) Temperature and water activity (*T* and a_w, Case 2); (c) Temperature and dissolved CO_2 (*T* and CO_2, Case 3); and, (d) All three factors (*T*, a_w and CO_2, Case 4.1). The values for the storage conditions used were $T = 4\ °C$, $a_w = 0.98$, and $CO_2 = 2650$ ppm. The shelf-life predicted by these models reflected the variability in the initial microbial load and the model parameters (see Table 3).

(b)

Figure 5, continuation. Distribution of the predicted shelf-life for meat based on the growth of *Lactobacillus sakei* and reaching 10^6 cfu/g as the shelf-life endpoint. The predictive microbiology models considered one or more storage conditions. (a) Temperature (*T*, Case 1); (b) Temperature and water activity (*T* and a_w, Case 2); (c) Temperature and dissolved CO_2 (*T* and CO_2, Case 3); and, (d) All three factors (*T*, a_w and CO_2, Case 4.1). The values for the storage conditions used were *T* = 4 °C, a_w = 0.98, and CO_2 = 2650 ppm. The shelf-life predicted by these models reflected the variability in the initial microbial load and the model parameters (see Table 3).

(c)

Figure 5, continuation. Distribution of the predicted shelf-life for meat based on the growth of *Lactobacillus sakei* and reaching 10^6 cfu/g as the shelf-life endpoint. The predictive microbiology models considered one or more storage conditions. (a) Temperature (*T*, Case 1); (b) Temperature and water activity (*T* and a_w, Case 2); (c) Temperature and dissolved CO_2 (*T* and CO_2, Case 3); and, (d) All three factors (*T*, a_w and CO_2, Case 4.1). The values for the storage conditions used were $T = 4$ °C, $a_w = 0.98$, and $CO_2 = 2650$ ppm. The shelf-life predicted by these models reflected the variability in the initial microbial load and the model parameters (see Table 3).
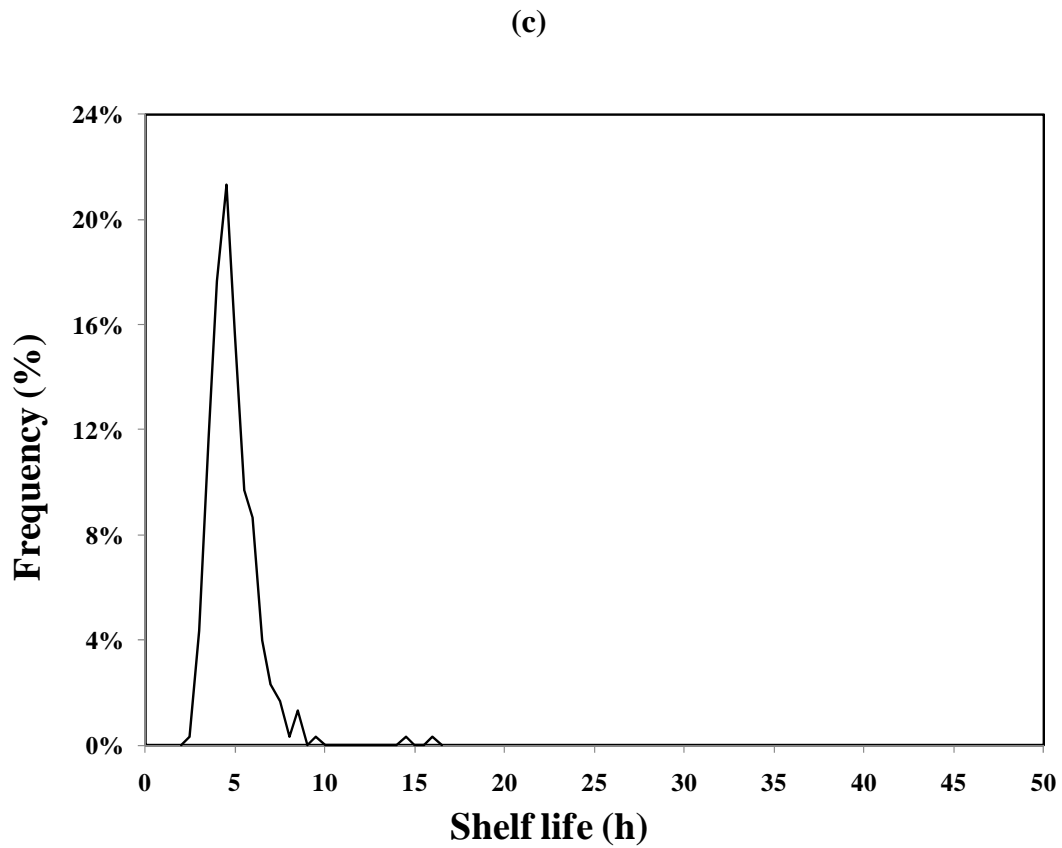
(d)

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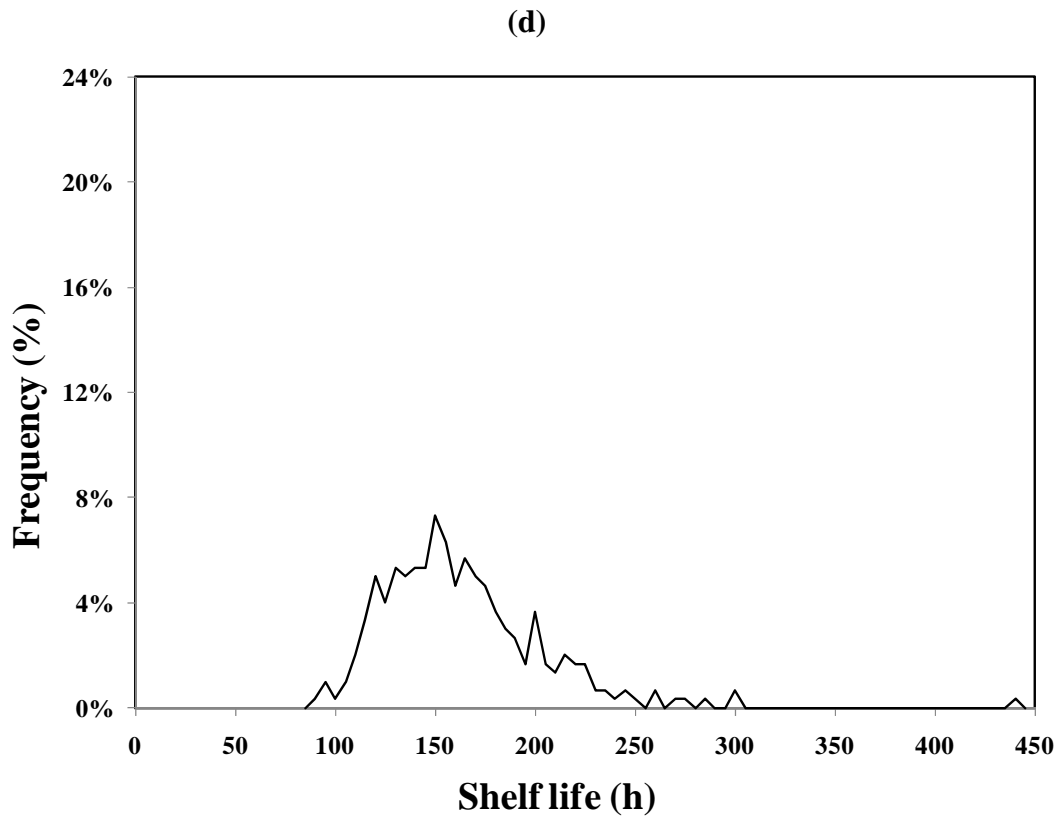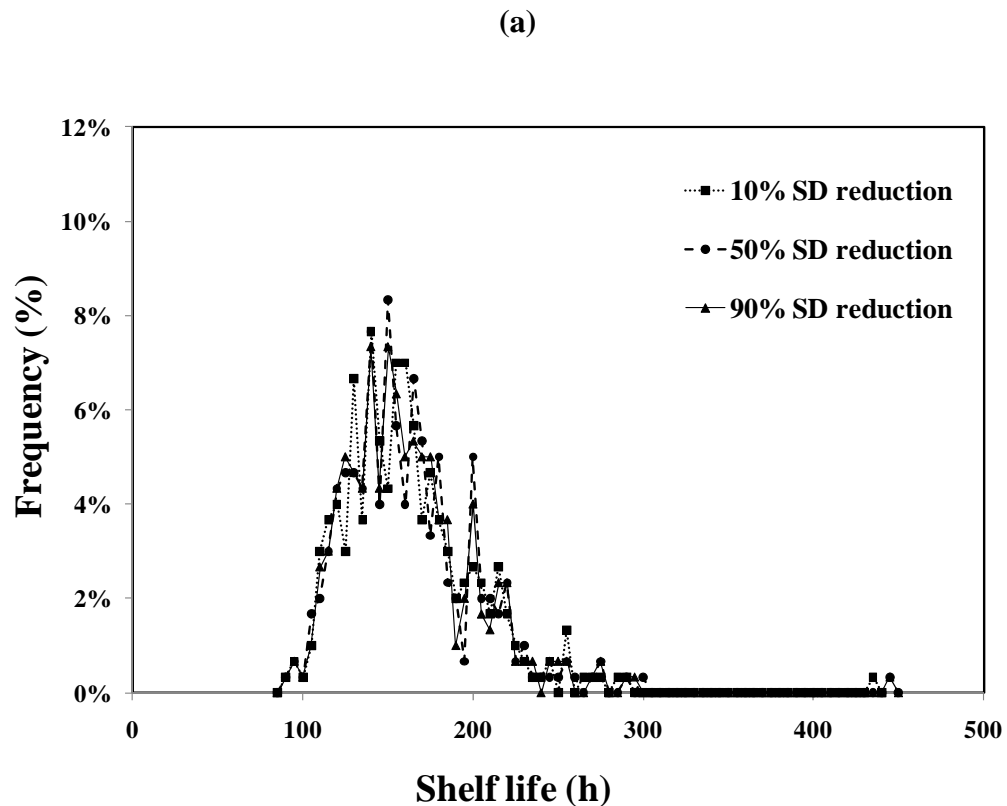
(a)

Figure 6. Effect of reducing by 10%, 50% and 90% the standard deviation (SD) value of the predictive microbiology model parameters (see Table 3, Case 4.2-4.5) used to estimate meat shelf-life based on the growth of *Lactobacillus sakei*. The model used considered storage temperature ($T = 4$ ℃), water activity ($a_w = 0.98$) and dissolved CO_2 ($CO_2 = 2650$ ppm). Variability in the shelf-life predicted reflected the variability in the initial microbial load and the model parameters (see Table 3). (a) Case 4.2: Reducing the SD for $a_{w\,min}$; (b) Case 4.3: Reducing the SD for T_{min}; (c) Case 4.4: Reducing the SD for b_4 and b_5; and, (d) Case 4.5: Reducing the SD for $CO_{2\,max}$.

(b)

Figure 6, continuation. Effect of reducing by 10%, 50% and 90% the standard deviation (SD) value of the predictive microbiology model parameters (see Table 3, Case 4.2-4.5) used to estimate meat shelf-life based on the growth of *Lactobacillus sakei*. The model used considered storage temperature ($T = 4$ °C), water activity ($a_w = 0.98$) and dissolved CO_2 ($CO_2 = 2650$ ppm). Variability in the shelf-life predicted reflected the variability in the in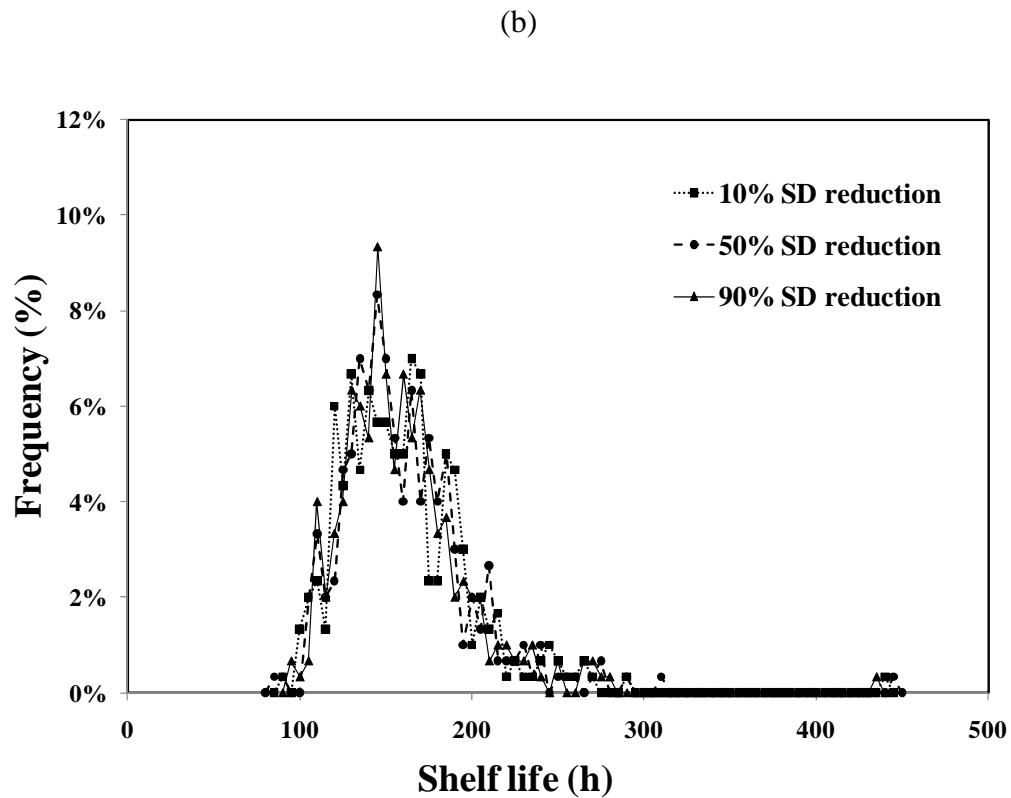itial microbial load and the model parameters (see Table 3). (a) Case 4.2: Reducing the SD for $a_{w\,min}$; (b) Case 4.3: Reducing the SD for T_{min}; (c) Case 4.4: Reducing the SD for b_4 and b_5; and, (d) Case 4.5: Reducing the SD for $CO_{2\,max}$.

(c)

Figure 6, continuation. Effect of reducing by 10%, 50% and 90% the standard deviation (SD) value of the predictive microbiology model parameters (see Table 3, Case 4.2-4.5) used to estimate meat shelf-life based on the growth of *Lactobacillus sakei*. The model used considered storage temperature ($T = 4\ °C$), water activity ($a_w = 0.98$) and dissolved CO_2 ($CO_2 = 2650$ ppm). Variability in the shelf-life predicted reflected the variability in the in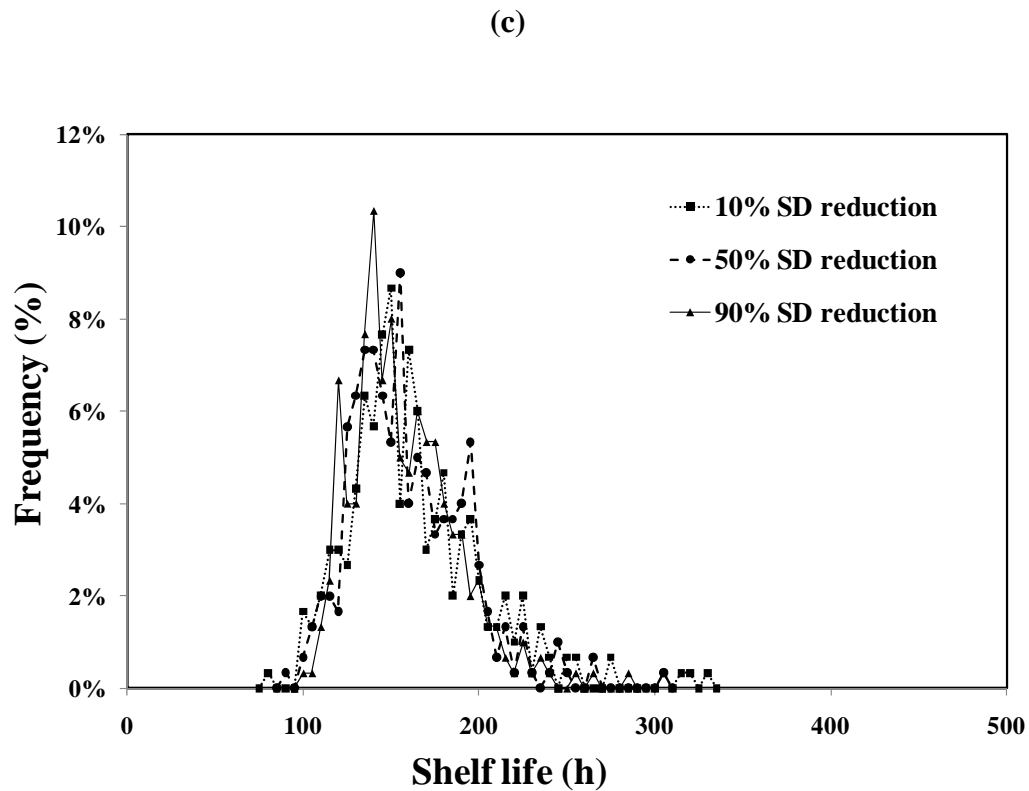itial microbial load and the model parameters (see Table 3). (a) Case 4.2: Reducing the SD for $a_{w\ min}$; (b) Case 4.3: Reducing the SD for T_{min}; (c) Case 4.4: Reducing the SD for b_4 and b_5; and, (d) Case 4.5: Reducing the SD for $CO_{2\ max}$.
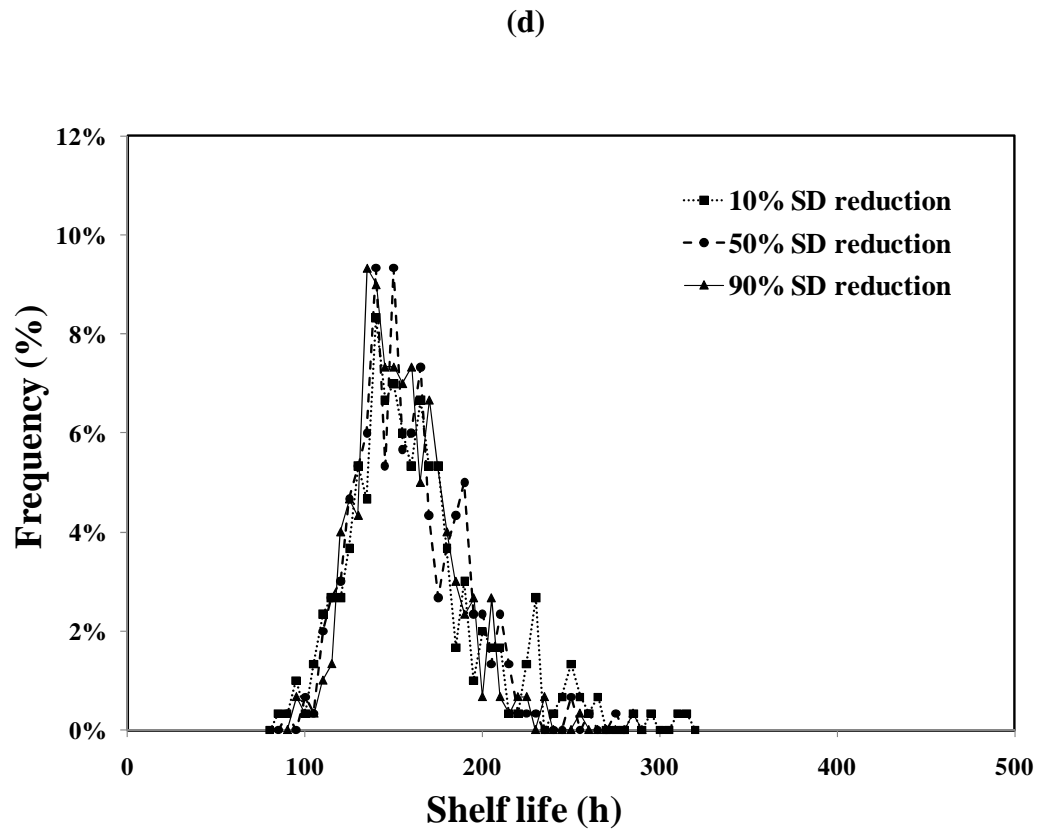
(d)

Figure 6, continuation. Effect of reducing by 10%, 50% and 90% the standard deviation (SD) value of the predictive microbiology model parameters (see Table 3, Case 4.2-4.5) used to estimate meat shelf-life based on the growth of *Lactobacillus sakei*. The model used considered storage temperature ($T = 4$ ℃), water activity ($a_w = 0.98$) and dissolved CO_2 ($CO_2 = 2650$ ppm). Variability in the shelf-life predicted reflected the variability in the initial microbial load and the model parameters (see Table 3). (a) Case 4.2: Reducing the SD for $a_{w\,min}$; (b) Case 4.3: Reducing the SD for T_{min}; (c) Case 4.4: Reducing the SD for b_4 and b_5; and, (d) Case 4.5: Reducing the SD for $CO_{2\,max}$.

(a)

Figure 7. Effect of reducing the standard deviation (SD) values of the predictive microbiology model parameters T_{min}, $a_{w\ min}$, b_4, b_5, and $CO_{2\ max}$ (see Table 3, Case 4.6) used to predict meat shelf-life based on the growth of *Lactobacillus sakei*. This model includes the storage conditions temperature (T = 4 °C), water activity (a_w = 0.98) and dissolved CO_2 (CO_2 = 2650 ppm). Variability in the shelf-life predicted reflected the variability in the initial microbial load and the model parameters (see Table 3). (a) Reducing SD values by 10%; (b) Reducing SD values by 50%; and, (c) Reducing SD values by 90%.

(b)

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(c)
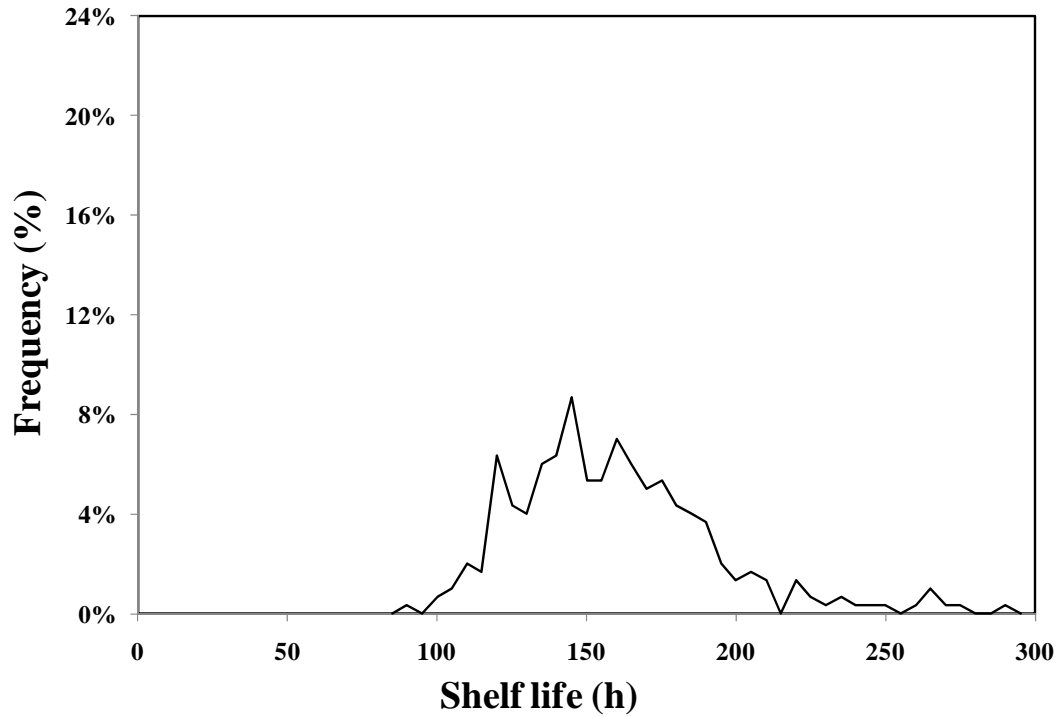

Figure 7, continuation. Effect of reducing the standard deviation (SD) values of the predictive microbiology model parameters T_{min}, $a_{w\ min}$, b_4, b_5, and $CO_{2\ max}$ (see Table 3, Case 4.6) used to predict meat shelf-life based on the growth of *Lactobacillus sakei*. This model includes the storage conditions temperature (T = 4 °C), water activity (a_w = 0.98) and dissolved CO_2 (CO_2 = 2650 ppm). Variability in the shelf-life predicted reflected the variability in the initial microbial load and the model parameters (see Table 3). (a) Reducing SD values by 10%; (b) Reducing SD values by 50%; and, (c) Reducing SD values by 90% (continued).

(a)

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(b)

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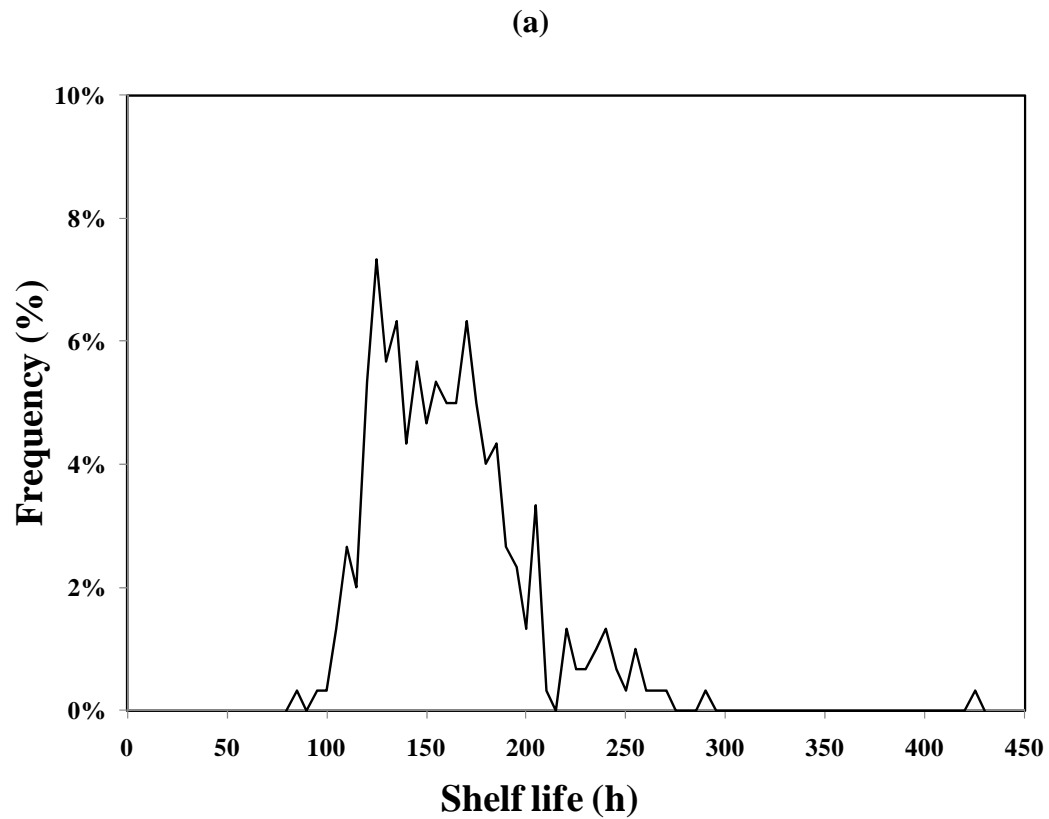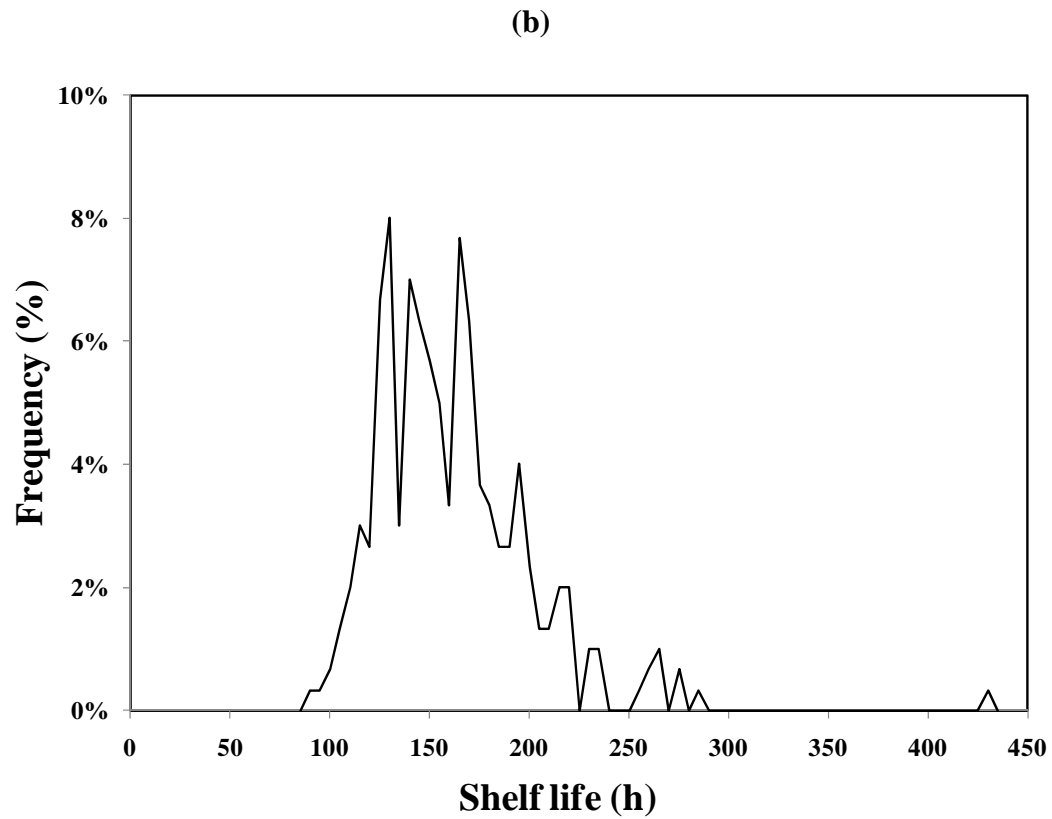
Impact of experimental parameter variability on thermal food processing decisions based on microbial quality and safety objectives

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Thermal food processing decisions

Abstract

Monte Carlo-type computer experiments can be used to evaluate the effect of the variability in model parameters for food safety and quality estimations. Procedures for inclusion in an undergraduate food process engineering course covering the assessment of uncertainty in thermal food processing decisions were developed using spreadsheets and operations found in the Excel^{TM} Analysis ToolPack. Published thermal decimal reduction time (D_T, $T = 110^oC$) and initial spore load (N_o, spores/container) level for *Clostridium botulinum* Type B in mushroom were used to estimate a thermal processing time (F_T). The survival probability (N) was the recommended value of 1 spore in 10^9 containers. Using reported mean values for the parameters D_T and N_o yielded $F_T = 5.96$ min. Unique combinations of generated N_o^* and D_T^* datasets were used to obtain the distribution for the spore survival probability and the associated percentage of under processing. Next, the coefficient of variation (CV) for the percentage of under processing when using 2 to 500 generated datasets was calculated to determine that 100 was an acceptable minimum number of datasets to estimate 9.6 min as a recommended thermal process considering the experimental variability of the parameters D_T and N_o and yielding a 10^{-9} failure probability with a 95% confidence. The predictive procedures were used also to assess the impact of reducing the standard deviation (*SD*) of both N_o and D_{110^oC} by 10%, 50%, and 90% yielding 8.6, 7.8 and 6.4 min, respectively, as a recommended thermal process at 110^oC with 95% confidence.

Keywords: undergraduate education, thermal processing, statistical variability, Monte Carlo simulation, Excel spreadsheets

Introduction

In the area of food process engineering education, recent reports have covered learning styles (Palou, 2006), comparison of knowledge gains and attitude changes using computer-based and face-to-face personal hygiene training methods (Fenton et al., 2006), construction of internet-assisted real-time experiments (Singh and Circelli, 2005), development and use of a web site with multimedia contents to assist unit operations courses (Tapia et al., 2005), use of web-based calculations to assist food engineering courses (Morales-Blancas et al., 2003; Morales-Blancas and Torres, 2004) and comparisons of food engineering education programs (Welti-Chanes and others 2002). No published studies reporting parameter variability considerations in food process engineering education were found.

Before implementing a process to eliminate a food safety hazard, an objective must be defined. The acceptable level of a hazard is expressed as a food safety objective (FSO), defined by the Codex Committee on Food Hygiene as "the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)" (Anonymous, 2004). In order to achieve an FSO, a process must be applied based on a performance criterion. The initial level of a hazard will change

during production and processing, distribution, storage, preparation, and food use (Stewart and others 2002). Therefore, a performance criterion can be defined by the equation:

$$H_0 - \Sigma R + \Sigma I \leq FSO \qquad (1)$$

where H_o is the initial, ΣR is the total decrease achieved by processing and ΣI is the total increase in the level of the hazard occurring after processing. In the case of the thermal processing of low acid foods, H_o is minimized through supplier selection and rejection of contaminated raw materials. ΣI is reduced to zero by preventing post contamination and growth of surviving spores, and ΣR is achieved by destroying the hazard by heat. Equation (1) expresses in an integrated manner, the entire process that must be followed to produce safe foods (Stewart et al., 2002; van Schothorst, 2005; van Schothorst, 1998).

Market forces are not always adequate incentives for food safety because of high testing costs and the wide array of risk agents and their hazard potential (Unnevehr and Jensen, 1999). The elimination of microbial spoilage and safety risks by thermal processing requires knowledge of the statistical distribution of those risks and the variability of the process applied (Lund, 1978; Halder and others 2007; Smout et al., 2000b; Lenz and Lund, 1977; Smout and others 2000a; Smout and others 2000c; Smout and others 2003). The same approach has been used when estimating the microbial shelf-life of refrigerated foods (e.g., Almonacid-Merino and Torres, 2009).

Processors face uncertainties when they use predictive models to make processing decisions because parameter values have variability that needs to be considered. Variability sources include processing temperature control, characterization of the raw material source such as microbial load, and the determination of the intrinsic properties of foods. Variability in these parameters cannot be avoided and must be considered in product safety and quality assessments. Unfortunately, parameter variability considerations are often not included when reaching process, storage and distribution decisions and this may reflect a deficiency in undergraduate food science education programs. When teaching the design of thermal food processes, the instructor should consider that engineering decisions are based on mathematical and also on statistical models describing the variability of thermal processing parameters. The option of using worst-case values is undesirable as it leads to higher processing costs and lower product quality (Guldas and others 2008).

The impact of the probability distribution of the parameters in a model can be assessed by Monte Carlo-type computer experiments. In thermal processing, a minimum processing time is required to ensure a desired microbial inactivation level while causing a minimum effect on product quality (Peck, 2006). Generally, both food-poisoning and food-spoilage microorganisms are considered when predicting the process time and temperature required (Smith and Cash, 1997). The determination of a heat sterilization protocol begins with the calculation of a

constant temperature processing time at a reference temperature T (F_T value) to achieve a desired thermal inactivation level (Peleg and others 2005). The concept of decimal reduction time at a constant temperature T (D_T) is often used to estimate the time required for the reduction in microbial load (van Asselt and Zwietering, 2006). Assuming first order reaction kinetics, D_T is the time required for 90% microbial inactivation or 90% quality attribute degradation at constant temperature T (Morales-Blancas and Torres, 2003a; Morales-Blancas and Torres, 2003b; Morales-Blancas and Torres, 2003c).

Clostridium botulinum is the most feared threat to public health in low-acid foods, and for this reason, the destruction of its spores has been used as the minimal criterion for heat processing. Initially, it was arbitrarily established that in low-acid foods, the minimum process should be at least as severe to reduce these spores by 12 logarithmic cycles (Guldas et al., 2008). This approach did not consider whether the initial microbial load in a specific processing batch was high or low (Figure 9a). For example, if the inactivation of *C. botulinum* in a given food has a $D_{121°C}$ of 0.21 min, the process time $F_{121°C}$ under the 12D concept was always the same, i.e., 2.52 (= 12 x 0.21) min (Stumbo et al., 1975; Stumbo, 1973; Sun, 2006).

The *12D* concept evolved into setting the probability for the survival of spores from a heat-resistant thermophile of public health importance, again typically *C. botulinum* (Figure 9b). This probability was set at 1 in 10^9 containers or less for the presence of microbial pathogens (Toledo, 2007; Pflug, 1987). This

strategy of setting a fixed endpoint microbial safety requirement was designed to reward efforts to reduce the initial contamination level (N_o) and thus reduce the required thermal processing time. In addition to cost savings, consumers benefitted from an improved food quality, particularly a higher retention of nutrients and eating quality. More recently, regulatory agencies have begun to require evidence that a process target be met with a certain probability considering the variability of the calculation parameters (Figure 9c). This requirement is typically set at 95% confidence interval (CI) or higher (Smout et al., 2000b; Javis, 1989; Fernandez et al., 1999; Rieu et al., 2007). This new regulatory strategy should be presented in food process engineering courses covering the calculation procedures for thermal processing.

The objective of this study was to use Monte Carlo simulations to estimate a process time ensuring that the process target (1 bacterial spore pathogen in 10^9 containers) is met with a 95% probability. To assess the impact of the variability in the initial spore numbers (N_o) and in the decimal reduction time (D_T), process times were estimated using the same mean values but with a 0, 10, 50 and 90% reduction of the reported standard deviation of these two parameters. The values thus obtained were compared with the process time determined on the basis of mean N_o and D_T values. These food process engineering and statistical methods were combined into an MS Excel set of calculations suitable for an undergraduate food process engineering course. Finally, this study focused on *C. botulinum* Type B

spores (Barker and others 2002; Sugiyama and Yang, 1975) in canned mushrooms, a product with high commercial importance in the USA and elsewhere. According to the U.S. Department of Agriculture, mushroom consumption reached 1.13 billion pounds in 2001 increasing by more than 21% since 1991. Most were sold as canned products and nearly 76% was purchased as retail products (Lucier and others 2003).

Methods

The procedures followed to analyze the effect of variability in the parameters N_o and D_T on the safety of a given thermal process are summarized in Figure 10. Computer generated values used in the Monte Carlo simulations are indicated using an asterisk. Excel implementation instructions can be obtained from the corresponding author.

Predictive model

The process time F_T for commercial food sterilization can be estimated as follows (Toledo, 2007):

$$SV = \frac{\log N_o}{\log N} = (\log N_o - \log N) \tag{2}$$

$$F_T = SV(D_T) = (\log N_0 - \log N) D_T \tag{3}$$

where:

SV = sterilization value or number of decimal reductions

N_o = initial spore load (spores/container)

N = probability of final spore load, target = 10^{-9} (spores/container)

F_T = thermal process time (min) at constant temperature

D_T = decimal reduction time at constant temperature T

A random number procedure was used to generate values for N_o (Notermans and others 1989) and D_T (Odlaug and others 1978) assuming normal and lognormal statistical distributions, respectively. The size of each generated dataset was equal to the number of samples used to determine the N_o and D_T values reported in the literature (Chu, 2009; Efron and Tibshirani, 1986). An important next step before accepting and using generated data is to develop a metric to identify unacceptable datasets (Chu, 2009). By defining an acceptable thermal process time error (0.1 min in this study), it was possible to define an acceptable error in the mean, minimum or maximum values for the initial spore load N_o. This was done as follows:

reported $N_o = N_o$ (4)

generated $N_o^* = \varepsilon N_o$ (5)

$$F_T^{reported} = SV\, D_T = [\log(\frac{N_o}{N})]D_T = (\log N_o - \log N)D_T \qquad (6)$$

$$F_T^{generated} = [\log(\frac{N_o^*}{N})]D_T = \left[\log(\frac{\varepsilon N_o}{N})\right]D_T = (\log \varepsilon + \log N_o - \log N)D_T \quad (7)$$

$$Error = \left|F_T^{reported} - F_T^{generated}\right| \le 0.1 \min \qquad (8)$$

$$Error = \left|D_T \log \varepsilon\right| \le 0.1 \min \qquad (9)$$

A similar procedure was used to define an acceptable error for D_T values:

$$\text{reported } D_T = D_T \tag{10}$$

$$\text{generated } D_T{}^* = \varepsilon D_T \tag{11}$$

$$Error = \left| D_T - \varepsilon D_T \right| \leq 0.1 \tag{12}$$

In the next step, a metric was defined to determine if the distribution of generated data was approximately equivalent to the distribution of the data reported in the literature. The metric used and based on normalized errors in the mean, minimum and maximum value for N_o and D_T values, was defined as follow:

$$metric = \left| \frac{(\mu_o - \mu_o{}^*)}{\mu_o} \right| f_1 + \left| \frac{(a_o - a_o{}^*)}{a_o} \right| f_2 + \left| \frac{(z_o - z_o{}^*)}{z_o} \right| f_3 \tag{13}$$

$$metric = \left| \frac{(\mu_o - \varepsilon \mu_o)}{\mu_o} \right| f_1 + \left| \frac{(a_o - \varepsilon a_o)}{a_o} \right| f_2 + \left| \frac{(z_o - \varepsilon z_o)}{z_o} \right| f_3 \tag{14}$$

$$metric = \left| 1 - \varepsilon \right| (f_1 + f_2 + f_3) \tag{15}$$

where:

$\mu_o, \mu_o{}^* \ (= \varepsilon \mu_o) =$ mean values for reported and generated data

$a_o, a_o{}^* \ (= \varepsilon a_o) =$ minimum values for the reported and generated datasets

$z_o, z_o{}^* \ (= \varepsilon z_o) =$ maximum values for the reported and generated datasets

f_1, f_2, f_3 $=$ weight factors (a subjective decision) for the normalized errors in the mean, minimum, and maximum value, respectively

The f_i values used in this study were 1, 0.5, and 2 for the mean, minimum, and maximum value, respectively. This reflected a strategy of providing relatively more importance to the normalized error in the maximum value of the generated data as compared to the mean and minimum value. Next, acceptable errors (ε) for N_o and D_T as determined by Equations (9) and (12), respectively, were used to determine an acceptable value for the metric using Equation (15).

The steps described above were followed to generate 500 (= S) datasets for N_o^* and D_T^* while keeping track of the number of datasets generated before finding one meeting the metric restriction. Only acceptable generated datasets for N_o^* (12 values each) and D_T^* (9 values each) were used in Monte Carlo simulations to obtain a distribution of the probability of *C. botulinum* spore survival (log N^*, 12x9 = 108 values) for a constant $\overline{F_T}$ calculated using the reported mean $\log \overline{N_o}$ and $\overline{D_T}$ values as follows:

$$\log N^* = \log N_o^* - \frac{\overline{F_T}}{D_T^*} \tag{16}$$

$$\overline{F_T} = \left(\log \overline{N_o} - \log N\right)\overline{D_T} \tag{17}$$

A spore survival log mean (*log N**) and a percentage of under processing value was estimated for each unique combination of the five hundred N_o^* and D_T^* datasets. Next, coefficient of variation (CV) values were calculated for 2, 3, 4 …, s = 500 estimated percentage under processing values to determine a recommended

sample size S (Efron and Tibshirani, 1986; Almonacid-Merino and Torres, 2009):

$$\overline{\mu_S^*} = \sum_{i=1}^{n=S} \frac{x_i^*}{S} \tag{18}$$

$$CV = \left[\sum_{i=1}^{n=S} \frac{\left(x_i^* - \overline{\mu_S^*}\right)^2}{S} \right]^{\frac{1}{2}} \tag{19}$$

Finally, the thermal processing time was increased (F_T^r) for each unique combination of the recommended number of N_o^* and D_T^* datasets so as to meet the probability of final spore load target (N = 10^{-9} spores/container) with a 95% confidence. These calculations generated a distribution of F_T^r values which was used to determine a recommended thermal processing time producing the required spore inactivation with 95% confidence.

Application example

An initial *C. botulinum* Type B spore load was referred to a typical canned mushroom product size (113.4 g = 4 oz). Based on the spore load information reported by Notermans and others (1989), the mean and standard deviation values for log N_o used in this study were -1.36 ± 0.87 with minimum -2.83 and maximum 0.08 log spores/container. Using D_T values reported by Odlaug and others (1978) yielded the following metric inequalities for the evaluation of generated datasets for

N_o and D_T values:

$$0 \leq metric_{N_o} \leq 393.2 \tag{20}$$

$$0 \leq metric_{D_{T=110C}} \leq 0.45 \tag{21}$$

An important aspect of probability distributions is that they represent either

uncertainty, i.e., the lack of perfect knowledge of the parameter value to be reduced

by further measurements, or variability, i.e., the true heterogeneity of the population

that is a consequence of the physical system and irreducible by additional

measurements (Nauta, 2002; Nauta, 2000; Akterian and others 1999). The latter can

be reduced by finding the sources of this heterogeneity. In the case of microbial

load, the contamination level heterogeneity could reflect differences in the

production conditions of suppliers, which would suggest that products with widely

different contamination levels should be processed separately. In the case of

decimal reduction time, this could reflect the aggregation of experimental

determinations of microbial thermal inactivation in different media. The

recommendation would be to determine this parameter in a single matrix,

preferably the product to be processed. Since both recommendations would

generate costs to the processor, it is important to evaluate the impact on the

recommended thermal process time of reducing process uncertainty and variability.

Therefore, in addition to determining a recommended number of generated datasets

(sample size, S) and a thermal process time F_T^r yielding a safe process with 95%

confidence, the impact of reducing the variability in N_o and D_T values was assessed as follows. Spore survival log mean (*log N**) and percentage of under processing values were estimated for each unique combination of the recommended number of $N_o{}^*$ and $D_T{}^*$ datasets, i.e., *S* times, assuming a 10%, 50% and 90% reduction in the reported standard deviation for N_o and D_T separately and for N_o and D_T at the same time. The thermal processing time was then increased (F_T^r) for each unique combination of the $N_o{}^*$ and $D_T{}^*$ datasets with reduced variability so as to meet the probability of final spore load target (N = 10^{-9} spores/container) with a 95% confidence. These calculations (repeated *S* times) generated a distribution of F_T^r values which was used to determine a recommended thermal processing time producing a safe products with 95% confidence. The objective of these calculations was to determine the impact of reducing the variability of these two parameters on the thermal process time required for a safe process.

Results and Discussion

Data transformations and verification of statistical distribution

A review of the literature showed that most authors report only summarized data, i.e., mean (μ) and standard deviation (σ) values, without explicitly stating the statistical distribution for the data reported. This required the generation of data assuming a certain probability distribution for the two parameters considered in this study. The metric used to accept or reject generated datasets provided a necessary

but not sufficient test to validate the assumed distribution form for each parameter.

Although this was not case for the data on initial spore load used in this study (Notermans et al., 1989), most authors assume a normal instead of lognormal distribution for microbial counts. If that is the case, it would be necessary to convert the reported normal mean (μ) and standard deviation (σ) to lognormal distribution values, $\hat{\mu}$ and $\hat{\sigma}$, respectively, as follows (Pereira, 2009):

$$\hat{\sigma} = \sqrt{\ln\left[\left(\frac{\sigma}{\mu}\right)^2 + 1\right]} \tag{24}$$

$$\hat{\mu} = \ln\mu - \left(0.5 \times \left(\hat{\sigma}\right)^2\right) \tag{25}$$

The minimum, mean and maximum number of repetitions to generate 500 approved datasets was 1, 1.982, and 6 for N_o values and 1, 1.314 and 4 for $D_{110\,°C}$ values, respectively. These low values suggest that the assumption of lognormal and normal distribution for these two parameters was correct (Pereira, 2009).

Impact of statistical variability on thermal processing time

The 500 approved datasets for N_o (12 values) and $D_{110\,°C}$ (9 values) were used with no repetitions in Monte Carlo simulations to generate 500 distributions of spore survival ($log\,N^*$) for a constant $\overline{F_T}$ applied, calculated using the reported

mean $\log \overline{N}_o$ and $\overline{D_T}$ values. Table 6a shows an example of the expected levels of spore survival (108 $log\ N^*$ values = 12 N_o^* x 9 D_T^* values) for a constant $\overline{F_T}$ based on reported mean $\log \overline{N}_o$ (Notermans et al., 1989) and $\overline{D_T}$ values (Odlaug et al., 1978). The distribution of these values highlights the risk of thermal process decisions based on mean values (Figure 11a). In this example, the probability of producing an unsafe product would be 55% and varied from 20.4 to 88.9% and a mean of 51.4% in the 500 distributions examined (Figure 12). The solution to this safety risk is to estimate thermal processing times considering the statistical variability of the reported N_o and D_T values. This was done for the 500 Monte Carlo simulations generating a distribution of thermal processing times ranging from 6.5 to 10.3 min. To comply with the current recommendation of a process time reaching the desired food safety target with 95% confidence (Figure 9c) resulted in a recommended thermal process time of 9.4 min (Figure 13).

Estimation of a recommended number of generated datasets (sample size)

A disadvantage of a Monte Carlo analysis is that the number of repetitive simulations necessary to obtain an acceptable accuracy level must be large (Floschet et al., 2003) and thus it is important to determine a recommended number that will result in acceptable results. The 500 distributions generated by the Monte Carlo simulations were used to determine a recommended number of generated datasets (sample size, S) for the estimation of thermal processing time (F_T).

Standard deviation (*SD*) and coefficient of variation (*CV*) for the percentage of

under processing values were plotted as a function of sample size, i.e., from 2 to

500. The *CV* decreased rapid until reaching 100 datasets (Figure 14). Therefore,

this sample size was considered sufficient for the application of generated N_o^* and

D_T^* values to predict spores survival log mean (*log N**) and the percentage of under

processing.

Determination of a process time considering parameter variability

During industrial food production, process parameters such as microbial

loads are highly variable (Corradini and others 2001). In addition, the benefits of

efforts to reduce the variability of the thermal inactivation parameters obtained in

laboratory experiments and under real production conditions must be assessed.

Knowledge of the variability of generated N_o^* and D_T^* values was used to estimate

a thermal process time (F_T^r) required to reach 10^{-9} spores/container with a 95%

confidence. The same dataset selected previously was used to demonstrate that

increasing thermal processing time from $\overline{F}_{110°C}$ = 5.96 min to 8.89 min increased

the probability of meeting the spore load target from 55 to 95% (Table 6b, Figure

11b). The same process repeated for 100 generated datasets as recommended to

obtain reliable results, yielded a frequency distribution of thermal processing times

meeting the desired inactivation of bacterial spores. This resulted in $F_{110°C}^r$ = 9.6

min as the recommended thermal processing time yielding safe product with 95%

confidence (Figure 15a), i.e., a value similar to the one obtained when using 500

generated datasets (9.4 min, Figure 13).

Increasing the requirement of microbial lethality to comply with new

public health standards will increase the degradation of nutrients and quality factors.

In response to this new regulatory demand, processors will have to find means to

reduce process time as much as possible. An alternative would be to use variable

retort temperature profiles (Erdogdu and Balaban, 2003; Almonacid-Merino and

others 1993a). An option explored in this study was to assess the impact of efforts

to reduce the variability in N_o and D_T values. Assuming a 10, 50, and 90%

reduction in the standard deviation of these values (Table 7), resulted in tighter

distributions of thermal process times to reach the desired inactivation level, i.e.,

10^{-9} spores/container when reducing the variability in N_o (Figure 15b), $D_{110°C}$

(Figure 15c) and both $D_{110°C}$ and N_o (Figure 15d). The recommended thermal

process time yielding a safe process with 95% confidence was 9.6 min before

reducing variability. The effect of reducing the variability in N_o (Figure 15b, Table

7a) values by 10, 50, and 90% resulted in recommended $F^r_{110°C}$ = 9.2, 8.8, and 8.6

min, respectively, while reducing the variability in D_T the recommended values

would be 9.4, 8.6, and 8.2 min (Figure 15c, Table 7b). The impact of reducing the

variability of both N_o and D_T on the thermal process time yielding a safe process

with 95% confidence the values would be 8.6, 7.8, and 6.4 min (Figure 15d, Table

7b), respectively. The latter value (6.4 min) is not very different from the value

calculated based on mean values and resulting in a 55% risk of under processing (5.96 min).

Conclusions

In this work, numerical computations of thermal processing times required to achieve a desirable surviving spore load probability were generated from Monte Carlo-type computer experiments incorporating the variability of two key parameters, initial microbial load and decimal reduction times. The high percentage of processes calculated based on average values for these two parameters not meeting the desirable surviving spore load probability supports the new recommendations from government agencies to incorporate statistical requirement that a food safety must be met with a high probability.

Table 6.

Monte Carlo simulation example for one log $N_o{}^*$ (12 values) and one $D^*_{110°C}$ (9 values) dataset for *C. botulinum* in Mushrooms to predict number of survival spores (log N^*, 12x9 = 108 values). Bold numbers indicate unsafe processing.

a. $\overline{F}_{110°C}$ = 5.96 min, based on reported mean log N_o and $D_{110°C}$ values

log $N_o{}^*$	$D^*_{110°C}$								
	0.97	0.68	1.05	0.82	0.54	0.77	0.84	0.52	0.98
-2.80	**-8.96**	-11.58	**-8.49**	-10.03	-13.79	-10.52	-9.92	-14.33	**-8.89**
0.60	**-5.56**	**-8.18**	**-5.09**	**-6.64**	-10.39	**-7.13**	**-6.52**	-10.93	**-5.49**
0.10	**-6.07**	**-8.69**	**-5.60**	**-7.14**	-10.90	**-7.63**	**-7.03**	-11.43	**-6.00**
-0.92	**-7.09**	-9.71	**-6.62**	**-8.16**	-11.92	**-8.65**	**-8.05**	-12.46	**-7.02**
-2.26	**-8.42**	-11.04	**-7.96**	-9.50	-13.25	-9.99	**-9.39**	-13.79	**-8.36**
-1.38	**-7.54**	-10.16	**-7.08**	**-8.62**	-12.37	-9.11	**-8.51**	-12.91	**-7.47**
-1.21	**-7.38**	-10.00	**-6.91**	**-8.45**	-12.21	**-8.94**	**-8.34**	-12.75	**-7.31**
-0.67	**-6.83**	-9.45	**-6.36**	**-7.90**	-11.66	**-8.40**	**-7.79**	-12.20	**-6.76**
-2.75	**-8.92**	-11.54	**-8.45**	-9.99	-13.75	-10.48	-9.88	-14.29	**-8.85**
-2.15	**-8.32**	-10.93	**-7.85**	-9.39	-13.14	-9.88	-9.28	-13.68	**-8.25**
-1.62	**-7.78**	-10.40	**-7.31**	**-8.86**	-12.61	-9.35	**-8.74**	-13.15	**-7.71**
-1.55	**-7.71**	-10.33	**-7.24**	**-8.79**	-12.54	-9.28	**-8.67**	-13.08	**-7.64**

b. $\overline{F}_{110°C}$ = 8.89 min required to meet $N = 10^{-9}$ spores/container with 95% confidence

log $N_o{}^*$	$D^*_{110°C}$								
	0.97	0.68	1.05	0.82	0.54	0.77	0.84	0.52	0.98
-2.80	-11.99	-15.90	-11.30	-13.60	-19.20	-14.33	-13.43	-20.00	-11.89
0.60	**-8.60**	-12.50	**-7.90**	-10.20	-15.80	-10.93	-10.03	-16.61	**-8.49**
0.10	-9.10	-13.01	**-8.40**	-10.70	-16.31	-11.44	-10.54	-17.11	-9.00
-0.92	-10.12	-14.03	-9.43	-11.73	-17.33	-12.46	-11.56	-18.13	-10.02
-2.26	-11.46	-15.37	-10.76	-13.06	-18.66	-13.80	-12.90	-19.47	-11.36
-1.38	-10.58	-14.48	-9.88	-12.18	-17.78	-12.91	-12.01	-18.59	-10.47
-1.21	-10.41	-14.32	-9.72	-12.02	-17.62	-12.75	-11.85	-18.42	-10.31
-0.67	-9.86	-13.77	-9.17	-11.47	-17.07	-12.20	-11.30	-17.87	-9.76
-2.75	-11.95	-15.86	-11.26	-13.56	-19.16	-14.29	-13.39	-19.96	-11.85
-2.15	-11.35	-15.26	-10.65	-12.95	-18.56	-13.69	-12.79	-19.36	-11.25
-1.62	-10.82	-14.72	-10.12	-12.42	-18.02	-13.15	-12.25	-18.83	-10.71
-1.55	-10.75	-14.65	-10.05	-12.35	-17.95	-13.08	-12.18	-18.76	-10.64

Table 7.

Effect of statistical variability of the prevalence of *Clostridium botulinum* (N_o) Type B spores and their decimal reduction time (D_{110^oC}) on the thermal processing time for canned mushrooms required to reach the desired inactivation level (10^{-9} spores/container). Calculations based on the recommended number of 100 generated datasets.

(a) Standard deviation reduction (mean ± SD)

Parameter	0%	10%	50%	90%
log N_o	-1.36 ± 0.87[1]	-1.36 ± 0.78	-1.36 ± 0.44	-1.36 ± 0.09
D_T	0.78 ± 0.17[2]	0.78 ± 0.153	0.78 ± 0.085	0.78 ± 0.017

(b) Processed time to produce safe food with > 95% confidence (min)

log N_o		9.2	8.8	8.6
D_T	9.6	9.4	8.6	8.2
Both		8.6	7.8	6.4

(1) Notermans and others (1989)
(2) Odlaug and others (1978)

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Figure 9. Effect of the food safety objective on the design of thermal preservation processes. (a) Original objective required the same 12 decimal reduction process for all producers; (b) Definition of a process endpoint of 1 spore survival in 1 billion containers rewarded efforts lowering the initial microbial load; (c) Consideration of the statistical variability in process parameters ensures that the food safety objective is met with high statistical confidence.

Figure 10. Monte Carlo methodology considering statistical variability in process parameters to ensure that a food safety objective is met with high statistical confidence.
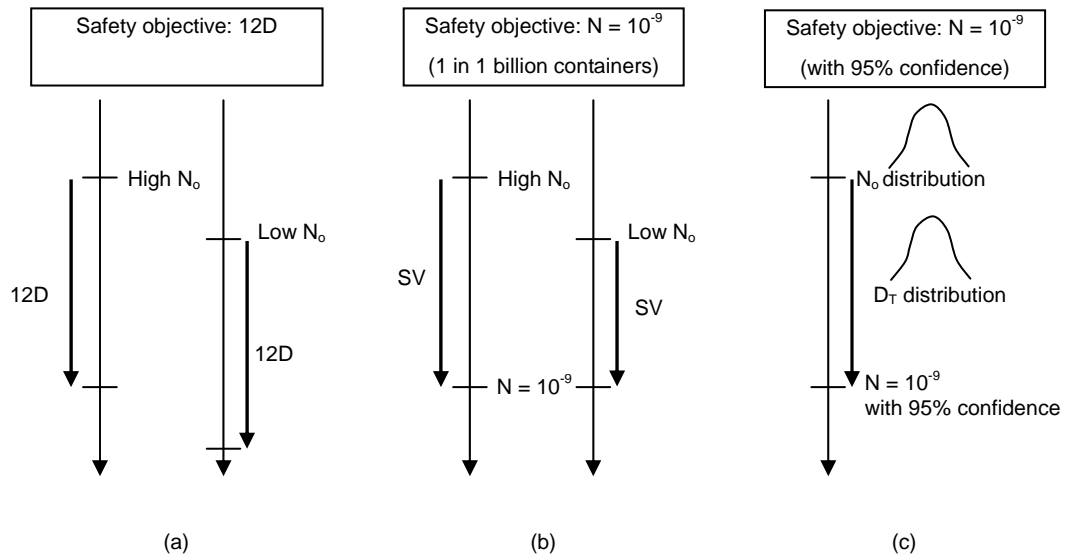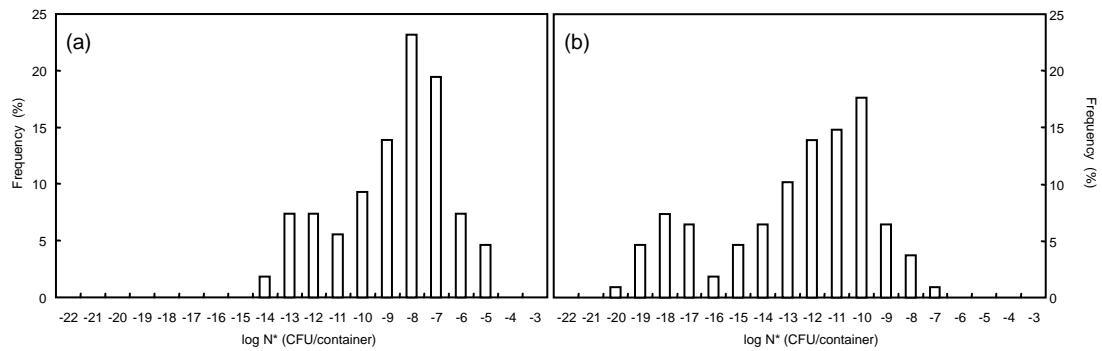
Figure 11. Probability distribution for *C. botulinum* spore survival ($log\ N^{*}$, CFU/container) for the same example of a dataset of generated N^{*}_{o} and $D^{*}_{110°C}$ values. (a) Spore survival values obtained for a thermal process time (= 5.96 min) based on the reported mean values for N_{o} and $D_{110°C}$; (b) Spore survival values obtained when the thermal process time was increased (= 8.89 min) to meet the process target (N = 10^{-9} spores/container) with 95% confidence.
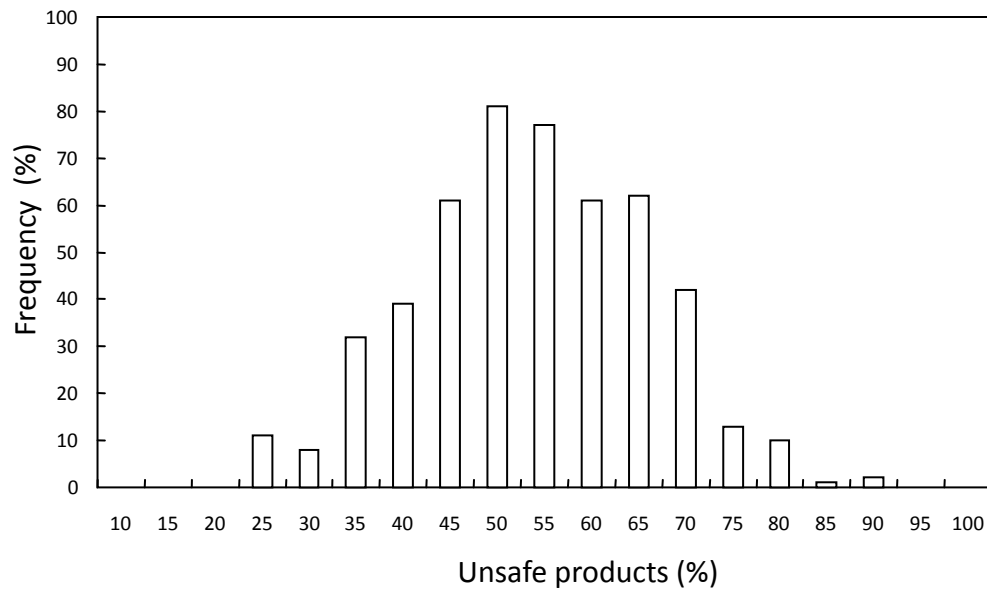
Figure 12. A distribution of the probability of unsafe products (%) based on 500 Monte Carlo simulations for a thermal process time (= 5.96 min) calculated using the reported mean values for N_o and $D_{110°C}$
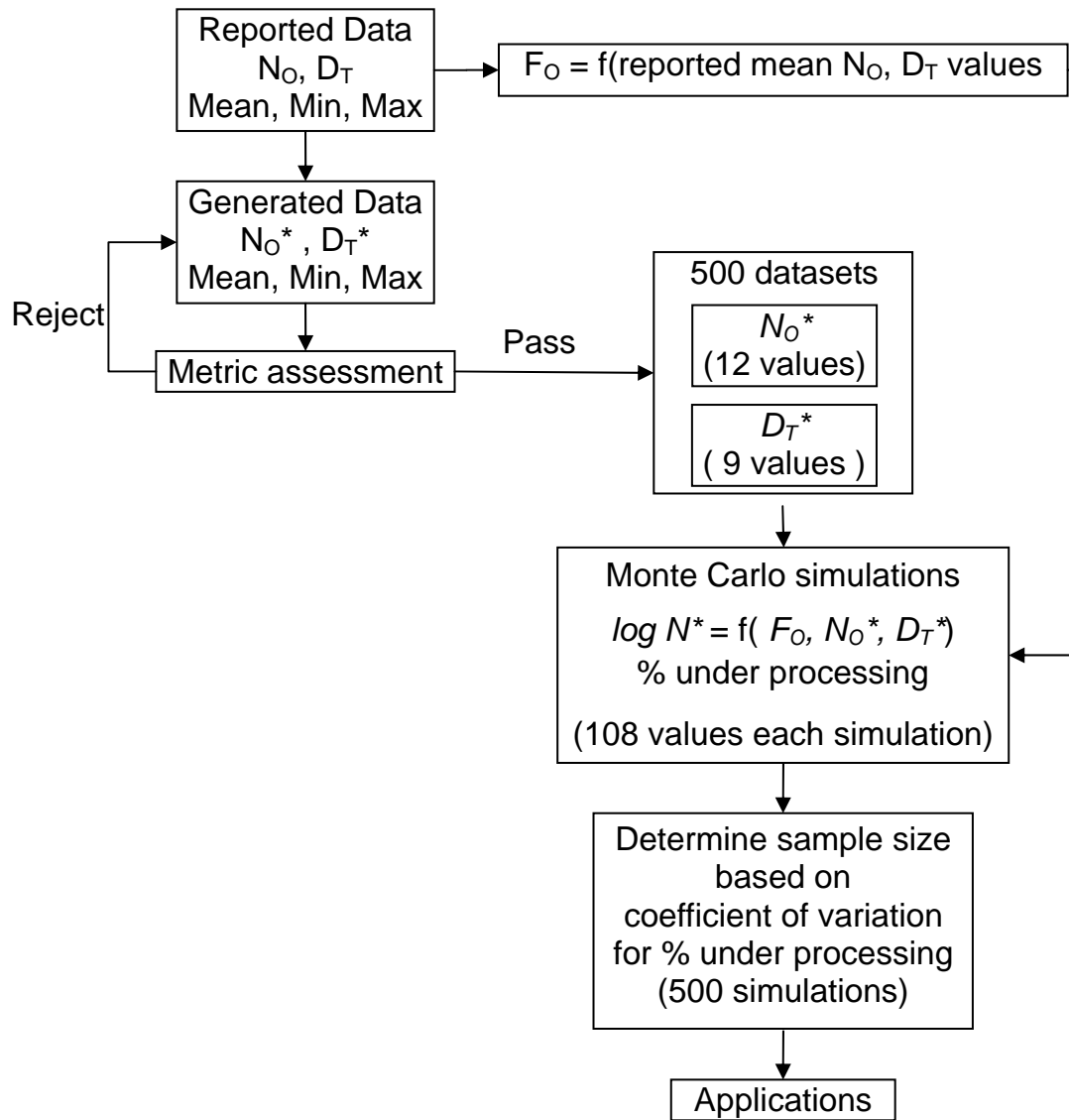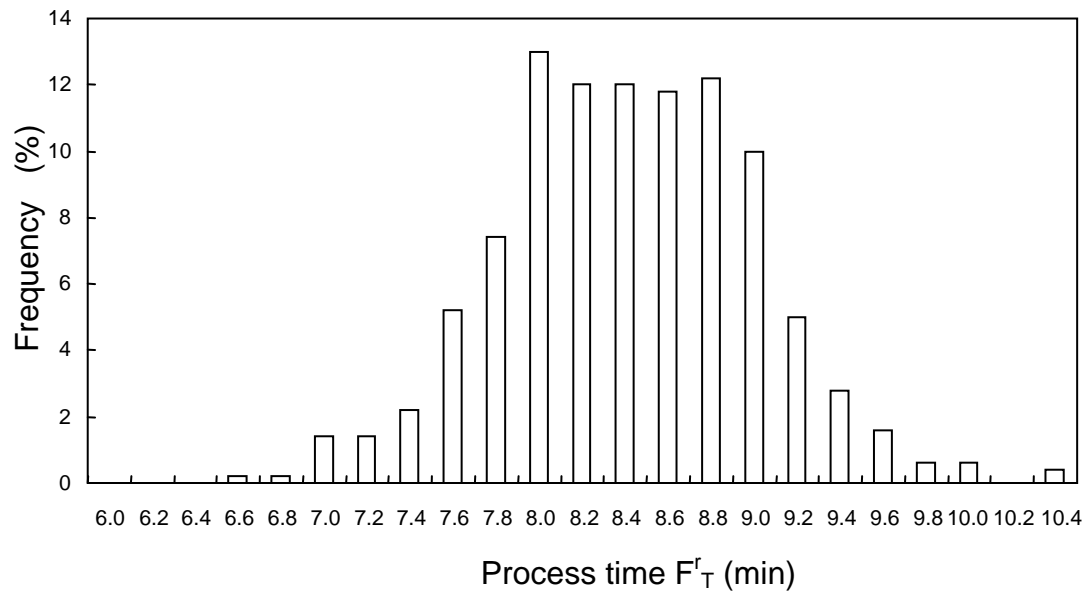
Figure 13. Probability distribution based on 500 datasets of processing times meeting the process target (N = 10^{-9} spores/container) with 95% confidence
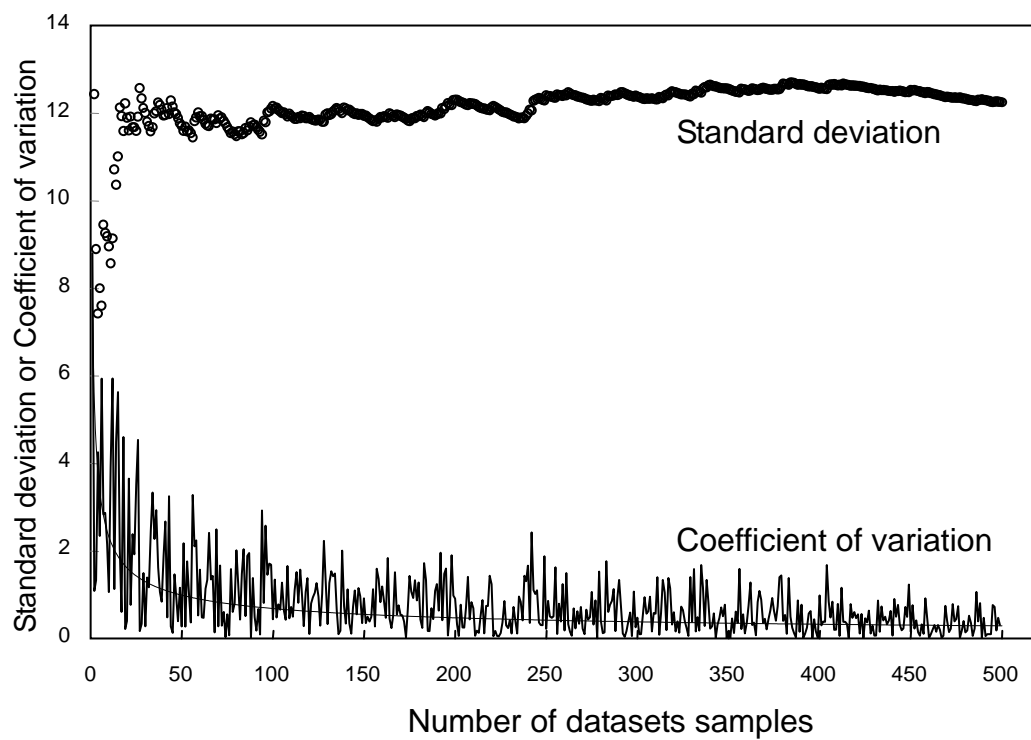
Figure 14. Determination of the recommended number of Monte Carlo simulations for the estimation of thermal processing time (F_T) considering the variability in the

reported spore load (N_o) and decimal reduction time (D_T) for *Clostridium botulinum* spores in mushrooms.
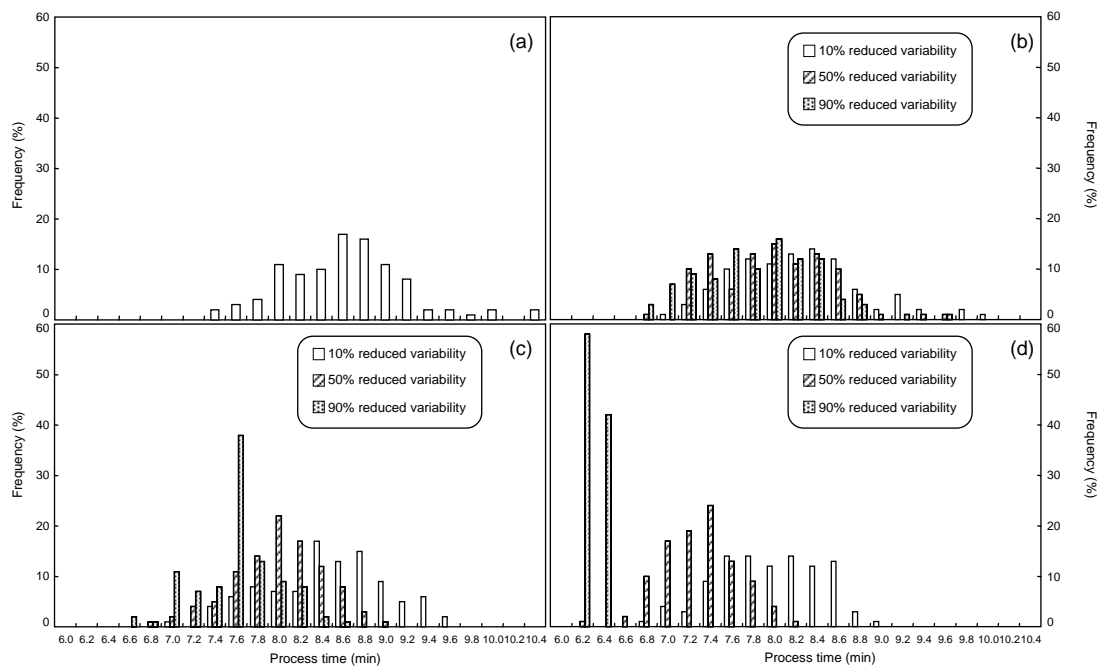
Figure 15. Distribution of the processing time reaching the desired inactivation level, i.e., 10^{-9} spores/container. Calculations based on the recommended number of generated datasets (100). (a) Original variability in N_o and D_T values ; (b) Reducing N_o variability by 10%, 50% and 90%; (c) Reducing D_T variability by 10%, 50% and 90%; (d) Reducing variability of both N_o and D_T by 10%, 50% and 90%.

Overall Conclusions

Predictive microbiology models are used in undergraduate food science programs to expose students to the effect of food properties and storage conditions on microbial shelf-life. As presented in this thesis, a significant educational improvement would be to use Monte Carlo procedures to estimate a microbial shelf-life with a specified degree of confidence and considering the variability of all model parameters. This can generate a rapid and cost-effective tool to reduce the risk of reaching consumers with unsafe or spoiled food products.

The variability of Ratkowsky-type model parameters for lag phase (λ) and exponential growth rate (μ_{max}) was used to estimate a probability distribution for the microbial shelf-life of meat. Random number generations for lognormal (initial microbial load) and normal distributed parameters (all other parameters) combined with Monte Carlo simulations were implemented using Microsoft Excel to determine a meat shelf-life probability distribution calculated using these predictive models. This allowed the determination of a recommended shelf-life considering the variability in microbial load and model parameters. The shelf-life value obtained considering the variability of the information and setting a 95% confidence interval (CI) (Smout and others 2000d; Smout et al., 2000b; Javis, 1989; Fernandez et al., 1999; Rieu et al., 2007) differed significantly from simpler calculations using only mean values for all parameters. This strategy of implementing in the form of Excel spreadsheets the generation of model parameter

values following known statistical distributions and Monte Carlo simulations for the generation of a shelf-life probability distribution should be presented in food process engineering courses covering estimations of microbial shelf-life. Excel implementation instructions can be obtained from the corresponding author.

The same Monte Carlo simulations-based approach can be used to explore the impact of the variability in multiple factors including microbial load of raw materials, food formulation, processing steps, packaging strategies including modified atmosphere packaging (MAP), as well as the conditions found during storage, shipping and distribution. Microbiology laboratory experiments to test all these factors and their expected variability would be complex and prohibitively expensive. Although the computer implementation of these models reduces this cost, their effectiveness depends on the determination of the values for several parameters under conditions at least similar to the application of interest and knowing the statistical distribution functions describing these experimental measurements.

Thermal processing has remained the foundation of the processed foods industry (Clark, 2002). Therefore, teaching of food thermal processing should inform students about new development including considerations of the variability in model parameters using the Monte Carlo procedures as described in this thesis. Monte Carlo simulations were used to estimate a thermal process time at constant temperature ensuring that the process target (1 bacterial spore pathogen in 10^9

containers) is met with a 95% probability. Numerical computations of thermal processing times required to achieve a desirable surviving spore load probability were generated from Monte Carlo-type computer experiments incorporating the variability of two key parameters, initial microbial load and decimal reduction times. The high percentage of processes calculated based on average values for these two parameters not meeting the desirable surviving spore load probability (55% in the example shown in this thesis) supports new recommendations from government agencies incorporating the requirement that the specified food safety target met is with a high probability.

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