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Modelling the effectiveness of pasteurisation

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6.1 Introduction: the role of predictive modelling

Processing of food products is designed in part to reduce or eliminate potentially pathogenic microorganisms which may cause serious illness if ingested. Consumers are now demanding fresher, less processed foods, and there is a move towards the development of minimally processed foods, which are protected by a series of intervention steps (or 'hurdles') (Leistner and Gorris, 1995). It is essential that the efficacy of these hurdles be assessed for each food product; however, the traditional approach of challenge testing is time consuming and labour intensive. Mathematical modelling of microbial survival and growth in foods provides a structured approach to ensure the safety of the food supply.

Thermal inactivation of microorganisms in static systems is usually described by the D - and z -value concepts as discussed below, with temperature generally held constant. The situation in canning operations or continuous flow systems such as high-temperature short-time (HTST) pasteurisation, ultra high temperature (UHT) and sterilisation processes is somewhat more complex, due to non-isothermal conditions, thus an integrated lethal effect approach is required (Kessler, 1986). In addition, the kinetics of inactivation in continuous systems differ from those of batch systems, since in the former systems there are additional factors such as pressure and shear forces which can influence microbial survival (Mackey and Bratchell, 1989; Fairchild *et al.*, 1994). As most modern food processes are continuous, it is necessary to have additional information on survival of microorganisms in these processes; however, few studies have been published on laboratory or pilot plant continuous flow systems (for review, see Fairchild *et al.*, 1994). Development of databases and models

for microbial behaviour in foods during processing and storage is an important part of predictive modelling, thus we initiated studies to develop models for the survival of selected food-borne microorganisms and milk enzymes during HTST pasteurisation. This chapter will present some of the basic concepts of modelling thermal inactivation. It will then describe how pasteurisation models have been developed using a pilot plant pasteuriser, followed by a summary of the models developed to date. Finally, a brief review of risk assessment will be presented, with details relating to the incorporation of pasteurisation models into the risk assessment framework.

6.2 The development of thermal models

The classical approach to thermal destruction of microorganisms assumes simple first-order reaction kinetics under isothermal conditions:

$$\frac{dS_t}{dt} = -k'S_t \quad 6.1$$

where S_t is the survival ratio (N_t/N_0 , where N_t is the number of cells at time t and N_0 the number of cells at time 0), and k' is the rate constant. Thus the number of surviving cells decreases exponentially:

$$S_t = e^{-k't} \quad 6.2$$

and when expressed as \log_{10} gives:

$$\log S_t = -kt \quad 6.3$$

where $k = k'/\ln 10$. The well-known D -value (time required for a 1-log reduction) is thus equal to $1/k$, where k is the slope (Fig. 6.1). The D -values can also be expressed as:

$$D\text{-value} = \frac{t}{\log N_0 - \log N_t} \quad 6.4$$

When $\log D$ -values are plotted against the corresponding temperatures, the reciprocal of the slope is equal to the z -value, which is the increase in temperature required for a 1-log decrease in D -value (Fig. 6.1 inset). The rate constant can also be related to the temperature by the Arrhenius equation:

$$k = N_0 e^{-E_a/RT} \quad 6.5$$

where E_a is the activation energy, R is the universal gas constant, and T is the temperature in K.

The food processing industry has enjoyed an enviable record of safety, thus the concept of exponential death of microorganisms has persisted, and is now considered accepted dogma. In spite of this, non-linear survival curves were reported for some bacteria almost 100 years ago (Moats *et al.*, 1971). The theoretical basis for assuming logarithmic behaviour for bacteria is based on the assumption that bacterial populations are homogeneous with respect to thermal

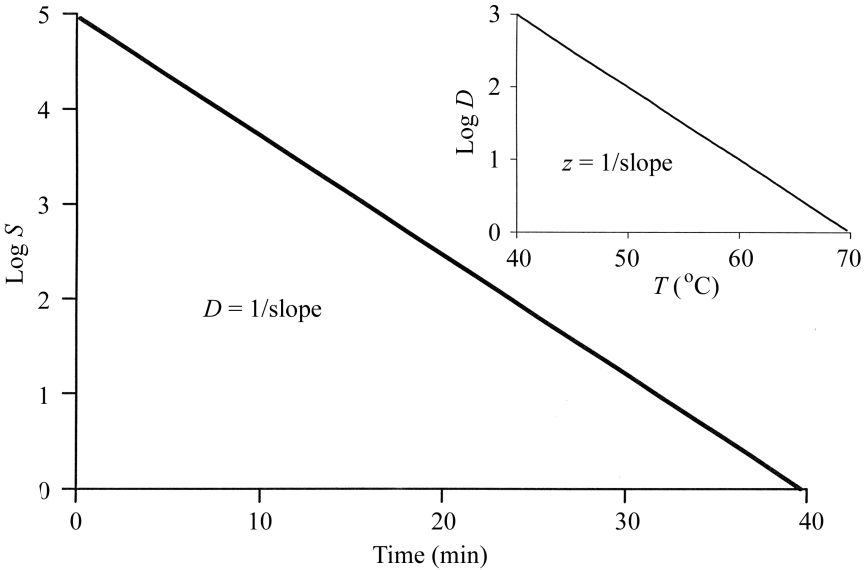


Fig. 6.1 Definition of the D - and z -values describing thermal inactivation kinetics.

tolerance, and that inactivation is due to a single critical site per cell (Moats *et al.*, 1971). Both of these assumptions have been questioned, and thus concerns have been raised regarding the validity of extrapolation of linear inactivation curves (Campanella and Peleg, 2001; Cerf, 1977). In general, there are two classes of non-linear curves: those with a ‘shoulder’ or lag prior to inactivation, and those which exhibit tailing. These two phenomena may be present together, or with other observed kinetics such as biphasic inactivation. A wide variety of complex inactivation kinetics have been reported, and several of these are shown in Fig. 6.2, which include shoulder (a), biphasic (b), sigmoidal (c), and concave (d).

Stringer *et al.* (2000) have assigned the possible explanations for non-linear kinetics into two classes: those due to artifacts and limitations in experimental procedure, and those due to normal features of the inactivation process. The first class encompasses such limitations as

- Variability in heating procedure
- Use of mixed cultures or populations
- Clumping
- Protective effect of dead cells
- Method of enumeration
- Poor statistical design.

The second class includes such situations as

- Possible multiple hit mechanisms
- Natural distribution of heat sensitivity
- Heat adaptation.

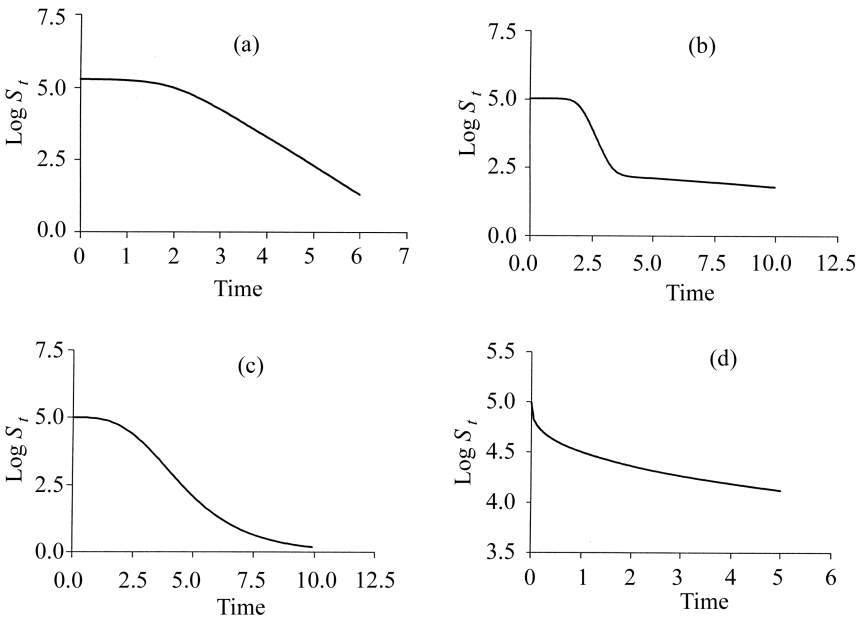


Fig. 6.2 Examples of non-linear thermal inactivation curves: (a) shoulder; (b) biphasic logistic; (c) sigmoidal; (d) concave.

These two classes roughly parallel two concepts reviewed by Cerf (1977) to explain tailing in bacterial survival curves. The first of these (the ‘mechanistic’ approach) also makes the assumption of homogeneity of cell resistance and proposes that thermal destruction follows a process analogous to a chemical reaction. In this approach, deviations from linearity are attributed mainly to artifacts; however, tailing is also related to the mechanism of inactivation or resistance. In the second (the ‘vitalistic’ approach) it is assumed that the cells possess a normal heterogeneity of heat resistance, thus survival curves should be sigmoidal or concave upward (Cerf, 1977).

Inactivation curves which deviate from simple exponential often have a lag or shoulder region prior to the exponential phase. This shape of inactivation curve is probably the one most commonly experienced by researchers. A simple linear model to account for this behaviour was developed by Whiting (1993):

$$\log N_t = \begin{cases} \log N_0 & \text{when } 0 < t < t_L \\ \log N_0 - \left(\frac{1}{D}\right)(t - t_L) & \text{when } t > t_L \end{cases} \quad 6.6$$

where t_L is the lag phase prior to inactivation. The Fermi equation, which is the ‘mirror image’ of the common logistic growth function, is also used for death curves which exhibit a shoulder (Pruitt and Kamau, 1993):

$$\log S_t = \log \left[\frac{1 + e^{-bt_L}}{1 + e^{b(t-t_L)}} \right] \quad 6.7$$

where b is the maximum specific death rate (Fig. 6.2a). When one wishes to include a secondary, more heat-resistant population, the resulting biphasic logistic function is (Whiting, 1993):

$$\log S_t = \log \left[\frac{F(1 + e^{-b_1 t_L})}{1 + e^{b_1(t-t_L)}} + \frac{(1-F)(1 + e^{-b_2 t_L})}{1 + e^{b_2(t-t_L)}} \right] \quad 6.8$$

where b_1 is the maximum specific death rate for the primary population and b_2 is the maximum specific death rate for the secondary population. Traditional D -values may be calculated as $2.3/b$ for each population. Lag phases are not always present, though this can be accounted for by setting the value of t_L to zero. An example of the output of this function is given in Fig. 6.2(b). Sigmoidal inactivation curves (Fig. 6.2(c)) can also be modelled using the asymmetric Gompertz function (Linton *et al.*, 1995):

$$\log S_t = Ce^{-e^{A+Bt}} - Ce^{-e^A} \quad 6.9$$

where A , B and C are coefficients. Another of the more common shapes of survival curves is the concave curve, which has no lag, and a single, tailing population (Fig. 6.2(d)). This function is best represented by the power law:

$$\log S_t = -\frac{t^p}{D} \quad 6.10$$

where p is the power. A concave curve is produced when $p < 1$.

One recent development in the modelling of bacterial survival is the use of distributions. This is based on the assumption that lethal events are probabilistic rather than deterministic, and that individual cells vary in their apparent thermal stability. The Weibull distribution is used in engineering to model time to failure, so it is appropriate for modelling bacterial inactivation. The distribution of survival times would then follow the probability density function (PDF) for the Weibull (solid line in Fig. 6.3):

$$\text{PDF} = \frac{\beta}{\alpha} \left(\frac{t}{\alpha} \right)^{\beta-1} e^{-(t/\alpha)^\beta} \quad 6.11$$

where α and β are parameters relating to the scale and shape of the distribution, respectively (vanBoekel, 2002). The survival curve is then the cumulative distribution function (CDF) (dotted line in Fig. 6.3):

$$\text{CDF} = e^{-(t/\alpha)^\beta} \quad 6.12$$

It can be easily seen that the CDF of the Weibull distribution is essentially a reparameterisation of the power law function (equation 6.10).

As mentioned earlier, non-isothermal conditions predominate in continuous food processes. Bigelow's (1921) model has been the non-isothermal standard model for the low-acid canned food industry for many decades. In this approach, the processing time F is determined by integrating the exposure time at various

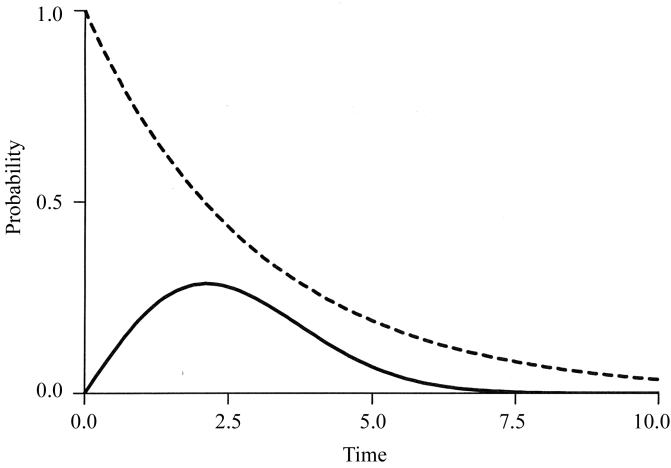


Fig. 6.3 Examples of the probability distribution function (solid line) and the cumulative distribution function (broken line) for a Weibull distribution.

temperatures $T(t)$ to time at a reference temperature T_{Ref} (Nunes *et al.*, 1993):

$$F = \int 10^{(T(t)-T_{\text{Ref}})/z} dt \quad 6.13$$

This model is considered to be an approximation of the Arrhenius model which is valid over a wide range (4–160°C) of temperatures (Nunes *et al.*, 1993):

$$\text{PE} = \frac{1}{t_0} \int_0^t e^{-(E_a/R)[(1/T)-(1/T_0)]} dt \quad 6.14$$

where

PE = integrated lethal effect, or pasteurisation effect

E_a = energy of activation (J mol^{-1})

R = 8.314 ($\text{J mol}^{-1} \text{K}^{-1}$)

T = temperature (K)

T_0 = reference temperature (345 K)

t = time (s)

t_0 = reference time (15 s).

The reference temperature (345 K or 72°C) and time (15 s) correspond to the International Dairy Federation standard for pasteurisation (Kessler, 1986).

It is often necessary for milk processors to demonstrate that the process they wish to use is effective in delivering the required lethal effect for the product and microorganism of concern. The integrated lethal effect is a useful concept, as it allows two or more processes which use different time/temperature combinations to be compared for efficacy against food-borne pathogens; however, there are few data available relating microbial survival to processing

conditions. This is of particular concern in the case of pasteurisation of milk, where the only accepted test for proper pasteurisation is the alkaline phosphatase (AP) test (Staal, 1986; International Dairy Federation, 1991). The relationship between AP inactivation and survival of food-borne pathogens is largely unknown, as is the response of AP to processing under alternative time/temperature combinations. In addition, data are lacking on the influence of continuous thermal processing on other milk enzymes which may be useful in monitoring thermal process above or below that required for pasteurisation (Griffiths, 1986; Andrews *et al.*, 1987; Zehetner *et al.*, 1996). These considerations prompted the initiation of work to develop mathematical models describing the survival of food-borne pathogens and selected milk enzymes during HTST pasteurisation.

6.3 Key steps in model development

6.3.1 Strains and culture conditions

Bacterial strains used in the various studies were maintained in glycerol at -20°C and propagated on Tryptic Soy Broth containing 0.6% (w/v) Yeast Extract (TSBYE). Strains were transferred twice into TSBYE at 30°C for 24 h, then inoculated into 20 litres of TSBYE at 30°C for 24 h. The cultures were concentrated to approximately 800 ml using a Pellicon filtration system with an HVMP000C5 $0.45\ \mu\text{m}$ filter and stored overnight at 4°C .

6.3.2 Pasteuriser design

The pasteuriser (Fig. 6.4) was designed to heat milk at a rate of $363\ \text{kg h}^{-1}$ from 2°C to temperatures as high as 80°C , and cool back to 4.5°C with 90% regeneration. Pressure on the milk leaving the regeneration section was boosted by means of a positive displacement pump to obtain a mean pressure differential of 41.4 kPa over the raw or feed side of the regeneration unit. The temperature of the milk in the pasteuriser was controlled using a Yokogawa YS170 process variable air-activated controller with three proportional settings in degrees Celsius.

6.3.3 Holding tubes

Holding tubes were constructed of 304 stainless steel with an internal diameter of 2.2 cm. The length of the holding tubes varied from 0.62 m (5.42 s) to as long as 17.23 m (63.93 s) depending on the residence time desired: a summary of holding times and lengths appears in Table 6.1.

Previous work of D'Aoust *et al.* (1987) estimated a Reynolds number of 10 500 for milk at 72°C in a 16.2 s holding tube, which is well above the critical minimum of 4000 required for turbulent flow. The minimum holding time was determined using a conductivity meter with the salt testing procedure set forth

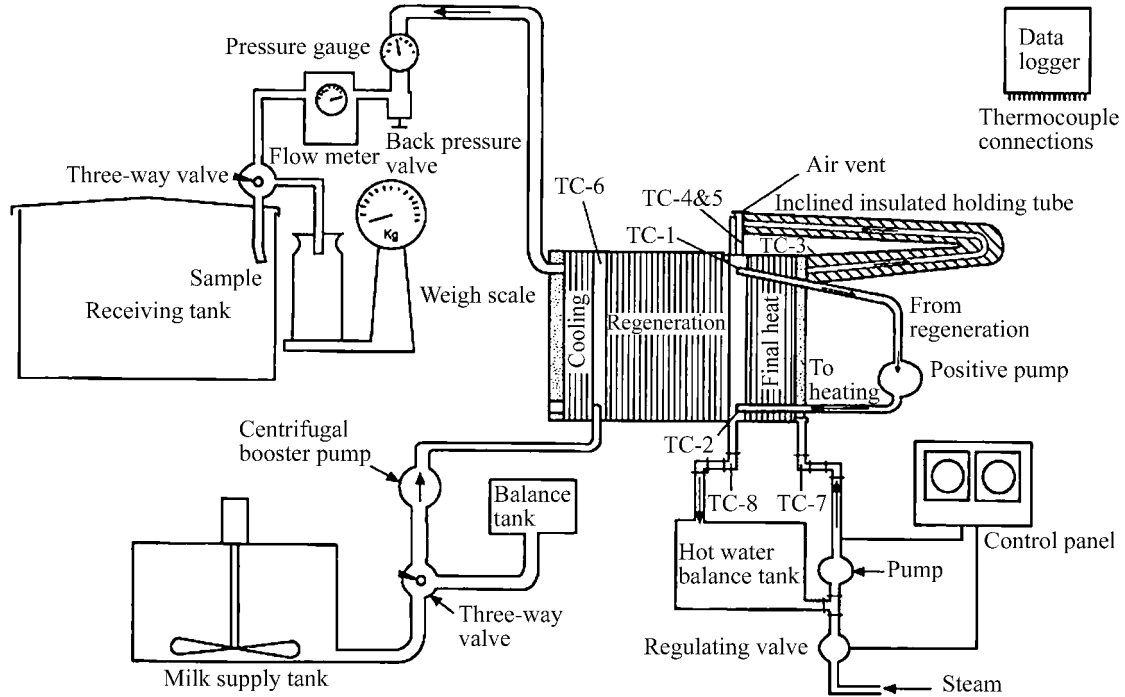


Fig. 6.4 Schematic diagram of the pilot-scale high-temperature short-time (HTST) pasteuriser from McKellar *et al.* (1994b) reprinted with permission from *Journal of Food Protection*. Copyright held by the International Association for Food Protection, Des Moines, Iowa, USA. Authors McKellar and Modler are with Agriculture & Agri-Food Canada, Guelph, Canada; authors Couture, Hughes, Mayers, Gleeson, and Ross are with Health Canada, Ottawa, Ontario, Canada.

Table 6.1 Holding times in APV-Crepaco Junior Paraflow Pasteuriser^a

Holding tube length (m)	Measured ^b minimum holding time (s)	Standard deviation	Maximum deviation	Corrected ^c holding time (s)
0.62	2.49	0.02	0.06	5.42
2.78	9.97	0.03	0.14	12.90
4.33	14.80	0.04	0.16	17.73
8.71	31.09	0.14	0.38	34.02
17.23	61.00	0.07	0.25	63.93

^a Data are from McKellar *et al.* (1994b) reprinted with permission from *Journal of Food Protection*. Copyright held by the International Association for Food Protection, Des Moines, Iowa, USA. Authors McKellar and Modler are with Agriculture & Agri-Food Canada, Guelph, Ontario, Canada; authors Couture, Hughes, Payers, Gleeson, and Ross are with Health Canada, Ottawa, Ontario, Canada.

^b Determined using the salt conductivity test ($n = 5$).

^c Obtained by adding 2.93 s to the minimum holding time to allow for feed and return port connectors (calculated from the total volume of the connectors assuming 80% efficiency).

by the International Association of Milk, Food, and Environmental Sanitarians, the Public Health Service and the Dairy Industry Committee (1992).

The minimum time, from the point of product entry into the regeneration section until collection at the receiving tank (excluding holding tubes), was estimated to be 57.8 s. The component times were as follows: regeneration (raw side) 5.00 s; heating, 4.63 s; regeneration (pasteurised side) to sampling valve 14.98 s; sampling valve to receiving tank 29.87 s; timing pump 3.28 s. Total residence time was obtained by adding the sum of the above times (57.8 s) to the 'corrected' holding tube times, reported in Table 6.1. This time was useful for determining when the product, for a particular heat treatment, had cleared the pasteurisation system.

6.3.4 Temperature monitoring

Temperatures were monitored using thermocouples (TC) at the following points (Fig. 6.4):

- TC 1 – end of regeneration (raw milk side)
 - TC 2 – after positive pump and before seating
 - TC 3 – end of heating
 - TC 4 – end of holding tube
 - TC 5 – end of holding tube
 - TC 6 – end of regeneration (pasteuriser side).
- } approximately same location

Thermocouples were inserted at the geometric centre of the product stream with the exterior stainless steel sheath being insulated with Imcolok Thermo Cel.

The thermocouples were connected to a Digistrip II recorder set to read at 2-s intervals and to print out all measurements at 1-min intervals. Data were

simultaneously collected with a portable computer using Telix V3.11 communications software. The thermocouples were calibrated at the Heat and Thermometry Laboratory, Physics Division, National Research Council of Canada, and were accurate within $\pm 0.2^\circ\text{C}$ when tested at 1°C intervals between 0 and 120°C . Prior to each run the thermocouples were also checked for error against ice-water.

6.3.5 Milk processing

Milk (3.5% milkfat) was obtained from the Greenbelt Farm of Agriculture Canada one day prior to experimentation. Approximately 1200–1600 litres of Holstein milk were pumped from the delivery truck to a 1670-litre Cherry-Burrell tank. When pathogen inactivation was being studied, milk was added also to two Mueller tanks each containing 200 litres of whole milk which had been previously heated to 80°C for 1 h, then cooled to 4°C . Each tank was then inoculated with 400 ml of culture concentrate (to approx. 10^8 cfu ml^{-1}), and 100 ml of the dye Fast Green FCF in distilled H_2O (1% w/v) was added as a marker for inoculated milk.

Processing consisted of starting at the highest temperature for a given holding tube, e.g. 80°C , and working down in 0.5 – 1.0°C increments until the lowest temperature had been achieved. This normally spanned the range of 5°C for a total of 6–10 samples per holding tube. Samples were taken only after the desired temperature had been achieved and maintained for a minimum of 3 min. When milk containing pathogens was being processed, temperatures were set using uninoculated milk from a bulk tank. Once a constant temperature had been established, flow was switched to the Mueller tank, and milk was sampled after the green dye had become apparent in the outflow. The flow rate was monitored using a mass flow meter, and was adjusted to 6.3 kg min^{-1} by manually controlling the variable speed drives of the feed and booster pumps.

6.3.6 Program development

The program to calculate PE was written in VisualBasic. PE for each section of the pasteuriser was determined by converting time at the indicated temperature to the equivalent time at the reference temperature using the empirical kinetic equation described by Kessler (equation 6.14). For each section of the pasteuriser, the cumulative lethal effect was determined using the trapezoidal rule (Gibaldi and Perrier, 1975), and expressed as PE. Total PE was calculated by adding the individual PE values for each section.

The stages in the HTST modelling program are given in Fig. 6.5. When a model was being derived, data from the TCs with the corresponding holding tube and residual enzyme activity or viable counts were input for each sample. Equipment calibration factors and residence times in the various parts of the system were input from separate configuration files. Lethal temperature was arbitrarily set at 60°C . A range for E_a/R was defined (usually 60 000 to 80 000)

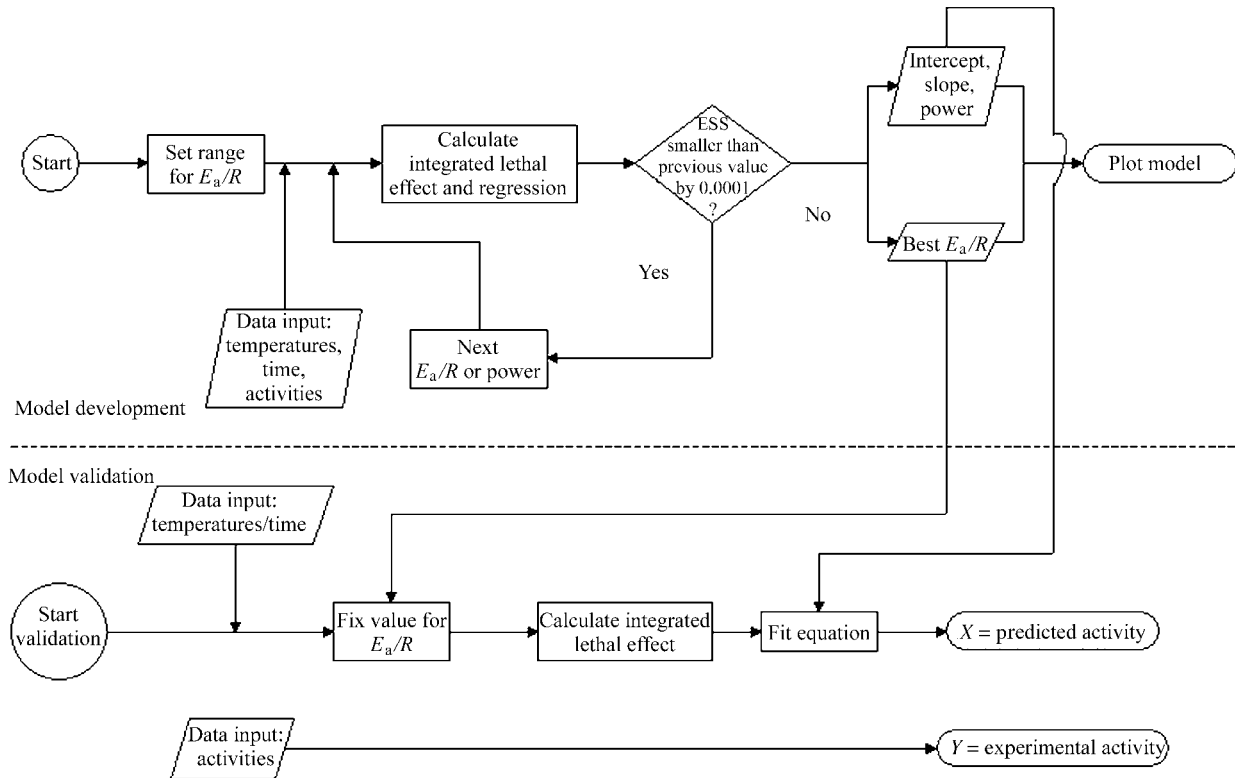


Fig. 6.5 Flow chart describing the development and validation of HTST models from McKellar *et al.* (1994b) reprinted with permission from *Journal of Food Protection*. Copyright held by the International Association for Food Protection, Des Moines, Iowa, USA. Authors McKellar and Modler are with Agriculture & Agri-Food Canada, Guelph, Canada; authors Couture, Hughes, Mayers, Gleeson, and Ross are with Health Canada, Ottawa, Ontario, Canada.

and the lowest value was selected. For each E_a/R , a total PE was calculated for each sample, and least-squares regression was performed between PE and \log_{10} % initial activity or initial cfu ml⁻¹. In order to improve the fit, a power law transformation (equation 6.10) of PE was incorporated in which a range of PE^c values were calculated ($c = 0.2$ to 1.0). The method of least squares was used (PE^c vs. \log_{10} % initial activity or initial cfu ml⁻¹) to obtain the best value of c by minimising the error sum of squares (ESS) for each value of E_a/R . ESS is defined as $(1 - r^2) \times \text{TSS}$, where TSS is the total sum of squares.

Values of E_a/R were incremented by 500, and the iteration was repeated as described above. The iterations were terminated when the new ESS value did not improve on the previous value by more than 0.0001. The final model consisted of the best E_a/R , slope, intercept and power (c). Output for each sample for further plotting was in the form $x = \text{PE}^c$, $y = \text{experimental data}$ (\log_{10} % initial activity or initial cfu ml⁻¹).

When an enzyme model was being validated, TC data, \log_{10} % initial activity values, and equipment configuration were input as described above (Fig. 6.5). The optimum E_a/R and c values were fixed, and PE^c was calculated for each data point. Intercept (a) and slope (b) values from the model were used to calculate predicted activity using the following equation:

$$\text{Predicted activity} = a + b \cdot \text{PE}^c \quad 6.15$$

and output was in the form $x = \text{predicted activity}$, $y = \text{experimental activity}$.

6.4 Models for key enzymes and pathogens

In model development, the parameter estimates for at least three trials were pooled for each milk enzyme or pathogen studied, and the mean parameter values are given in Table 6.2. Due to the importance of pasteurisation in milk processing, the first model was developed for AP (McKellar *et al.*, 1994b). There was also a need to develop models for milk enzymes which might be used to confirm processing at temperatures above or below pasteurisation. Higher

Table 6.2 Model parameters for inactivation of various milk enzymes and food-borne pathogens during HTST pasteurisation

Target	Trials	Intercept	Slope	Power	E_a/R ($\times 1000$)
Alkaline phosphatase	3	2.05	-4.05	0.50	66.5
γ -Glutamyl transpeptidase	3	2.00	-0.281	0.75	66.5
Lactoperoxidase	3	2.12	-0.096	0.75	59.0
Catalase	3	1.94	-2.65	0.50	82.0
α -L-fucosidase	3	1.87	-17.6	1.00	39.8
<i>Listeria innocua</i>	5	1.86	-24.9	0.80	59.5
<i>Listeria monocytogenes</i>	3	1.68	-18.4	0.80	48.5
<i>Enterobacter sakazakii</i>	3	2.31	-24.4	0.65	59.5

temperatures (at least 75°C) are appropriate for processing of more viscous dairy products such as ice-cream mix. Lactoperoxidase (LP) and γ -glutamyl transpeptidase (TP) are two naturally occurring milk enzymes which are inactivated at higher temperatures, and models were developed for these enzymes (McKellar *et al.*, 1996). Temperatures below pasteurisation of 63–65°C (termed sub-pasteurisation or thermisation) are used to extend the storage life of bulk milk. Models have also been developed for catalase (CA) (Hirvi *et al.*, 1996) and α -L-fucosidase (FC) (McKellar and Piyasena, 2000) which are appropriate for sub-pasteurisation temperatures. Parameter values for all enzyme models are found in Table 6.2.

Validation experiments were done for AP, LP, TP and CA using data from trials not used to develop the original models (procedure in Fig. 6.5). Since there was generally close agreement among the three trials for all these enzymes, a good relationship was obtained between predicted and experimental activities, with r^2 values ranging from 0.735 to 0.993 (McKellar *et al.*, 1994b, 1996; Hirvi *et al.*, 1996).

Survival models for several food-borne pathogens have also been derived. *Listeria innocua*, a non-pathogen, is often used as a substitute for *L. monocytogenes* in situations (such as food processing environments) where it would be undesirable to introduce pathogens (Fairchild and Foegeding, 1993). A model developed for *L. innocua* (Table 6.2) was shown to underpredict inactivation of *L. monocytogenes*, thus predictions are ‘fail-safe’ (Piyasena *et al.*, 1998). *Enterococcus faecium*, a non-pathogen, is also used as a model organism for pathogens, particularly in Europe (Gagnon, 1989). The inactivation curve for this microorganism deviated strongly from linearity, and there were large inter-trial variations. Thus a random coefficient model using the biphasic logistic function (equation 6.8) was used to fit the data (Ross *et al.*, 1998). The average $\ln D$ -values for the two populations were 0.825 and 2.856.

One of the more interesting target microorganisms examined in these studies was *Enterobacter sakazakii*, an ‘emerging’ pathogen found to contaminate infant formula. It was reported by Nazarowec-White and Farber (1997b) that 0–12% infant formula samples found on the Canadian retail market (from five different companies) contained *E. sakazakii*. Taxonomy and microbiology of this microorganism were described by Nazarowec-White and Farber (1997a) in their review on *E. sakazakii*. Model parameter values (Table 6.2) were generated for this pathogen from three independent trials, and the resulting regression lines are shown in Fig. 6.6 (Nazarowec-White *et al.*, 1999).

The power values listed for the various enzymes and pathogens (Table 6.2) give an indication of the extent of non-linearity of the inactivation curves. As described above, fitting with the power law function is appropriate for concave (tailing) inactivation curves when the power value is less than 1.0. In all cases except one, power values were less than 1.0, suggesting that, even in a continuous flow system, inactivation kinetics for many milk enzyme and food-borne pathogens deviate substantially from the linear. This supports the general observation made earlier that few, if any, survival curves are truly linear,

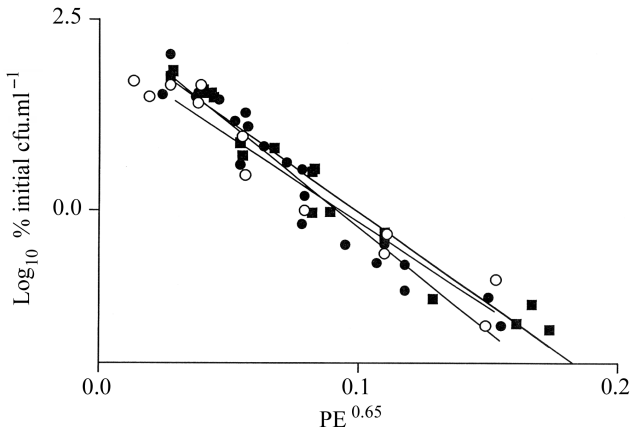


Fig. 6.6 Linear models derived from three HTST trials with *Enterobacter sakazakii*. Data are from Nazarowec-White *et al.* (1999) reprinted with permission from *Food Research International*. Copyright held by Elsevier Science Publishers.

suggesting that a first-order model for thermal inactivation is inadequate. A more mechanistic approach is clearly required to establish standard modelling procedures for thermal inactivation of milk enzymes and pathogens in a continuous flow system.

The parameter estimates from Table 6.2 were used to develop a stand-alone software (PasTime) which was designed to provide users with a simple method for calculating log reductions of milk enzymes and pathogens achieved by time-temperature combinations specified by the user (McKellar *et al.*, 1994a). Provision was also made to allow the user to enter calibration data (i.e. holding tube times and pasteurisation efficiency). The program is available free from the author.

6.5 Modelling and risk assessment

Historically the production of safe food has been based on numerous codes of practice and regulations enforced by various governing bodies worldwide. With the increased concern regarding the existence of microbial hazards in foods, a more objective approach is warranted, which has led to the introduction of the Hazard Analysis Critical Control Point (HACCP) system. HACCP as a tool for safety management consists of two processes: building safety into the product and exerting strict process control (Notermans and Jouve, 1995). The principles of HACCP have been set out by the Codex Alimentarius Commission (CODEX, 1993) and consist of seven steps: hazard analysis, determination of Critical Control Points (CCP), specification of criteria, implementation of monitoring system, corrective action, verification, and documentation (Notermans *et al.*, 1995). HACCP processes as defined for various food

products are often based on qualitative information and expert opinion. Moreover, the microbiological criteria underlying HACCP are poorly understood or defined (Buchanan, 1995).

The concept of risk assessment as defined by the Food and Agriculture Organisation and the World Health Organisation (FAO/WHO, 1995) provides a more quantitative approach to food-borne hazards. Quantitative risk assessment (QRA) is the scientific evaluation of known or potential adverse health effects resulting from human exposure to food-borne hazards (Notermans and Jouve, 1995). It consists of four steps: hazard identification, exposure assessment, dose response assessment, and risk characterisation (Fig. 6.7) (Notermans and Teunis, 1996). QRA is also considered to be part of the larger concept of risk analysis, which includes, in addition, risk management and risk communication steps (Fig. 6.7) (Notermans and Teunis, 1996). Risk assessment specifically supports step 3 of HACCP (Fig. 6.7) (Notermans *et al.*, 1995). The relationship between HACCP and risk assessment has not always been completely clear. For example, both processes start with the identification of hazards. Risk assessment is intended to provide a scientific basis for risk management, while HACCP is a systematic approach to the control of potential hazards in food operations (Foegeding, 1997). Thus, risk assessment concerns the overall product safety, while HACCP enhances overall product safety by assuring day-to-day process control (Foegeding, 1997). The view of risk assessment being associated with one step of HACCP may be a limited one. In a contrasting view, both HACCP and risk assessment are encompassed in risk analysis, with HACCP representing one management strategy (Fig. 6.7) (Foegeding, 1997).

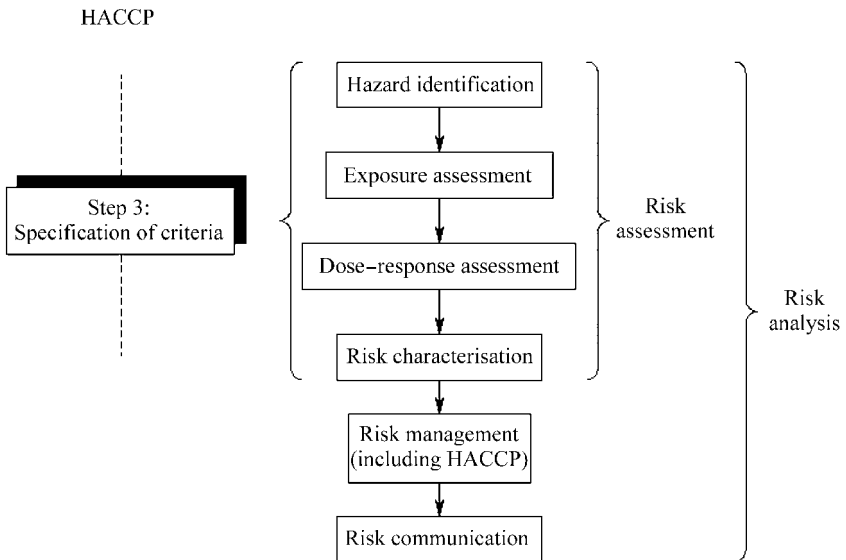


Fig. 6.7 Comparison of HACCP, risk analysis and risk assessment.

The recent ratification of the World Trade Organisation (WTO) agreement will have a major impact on the development of new approaches for the regulation of food. Countries will be encouraged to base their procedures on Codex standards and guidelines to maintain and enhance safety standards (Hathaway and Cook, 1997). This will lead to the development of harmonised risk assessment and risk management frameworks, providing input into HACCP, which is the primary vehicle for achieving enhanced food safety goals (Hathaway and Cook, 1997). As the use of HACCP increases, there will be a need for a clear understanding of the relationship among HACCP, microbiological criteria, and risk assessment (Buchanan, 1995). Regulators will be called upon to participate in all aspects of HACCP development, in particular to establish public health-based targets, elucidate microbiological criteria, develop improved techniques in microbiological risk assessment, and develop the means for evaluating the relative performance of HACCP systems (Buchanan, 1995). Harmonisation of international rules will clearly require standardised approaches (Lammerding, 1997).

In order to understand the role of predictive microbiology in risk assessment, it is necessary to further examine the various steps in more detail (Lammerding, 1997). Hazard identification involves the identification and characterisation of biological hazards that may be present in foods. Exposure assessment refers to the qualitative and/or quantitative evaluation of the likely intake of the biological agent. Dose–response assessment refers specifically to the determination of the number of microorganisms ingested, and the frequency and severity of adverse health effects. Risk characterisation is the qualitative and/or quantitative estimation of the probability of occurrence and severity of known or potential adverse health effects in a given population.

Mathematical modelling can have the greatest influence on the exposure assessment and dose–response assessment steps. Implicit in the concept of exposure assessment is the influence of processing and environmental factors on the survival and growth of food-borne pathogens. Mathematical models can predict the extent of impact of unit operations on the numbers of microorganisms, which in turn determines the exposure (Buchanan and Whiting, 1996). Specific mathematical functions to quantitate microbial growth and death can be incorporated into risk assessments (Buchanan and Whiting, 1996; McNab, 1997; Walls and Scott, 1997; vanGerwen and Zwietering, 1998). For example, the Gompertz function is used to evaluate growth parameters:

$$\log N(t) = A + C(e^{-e^{-B(t-M)}}) \quad 6.16$$

where $N(t)$ is the number of cells at time t , A is the asymptotic count as t decreases to zero, C is the difference in value between the upper and lower asymptotes, B is the relative growth rate at M , and M is the time where the absolute growth rate is maximum (Buchanan and Whiting, 1996). Thermal death models can be used to establish the D -value for a microorganism (equation 6.4). Much information on microbial growth and survival has been documented, and the resulting predictive software packages such as Food MicroModel have been

used to predict the influence of food composition and environmental conditions on growth and survival of potentially hazardous microorganisms (Panisello and Quantick, 1998). Models can therefore be used to develop CCPs, and show where data for risk assessments are missing (Baker, 1995). In addition, models can support regulations and optimise product formulations and support process control (Baker, 1995). Mathematical modelling can also support quantitation in dose–response assessment. The beta Poisson distribution model for dose–response is (Buchanan and Whiting, 1996):

$$P_i = 1 - \left(1 + \frac{N}{\beta}\right)^\alpha \quad 6.17$$

where P_i is the probability of infection, N is the exposure, and α and β are coefficients specific to the pathogen. One is cautioned in the use of mathematical models for quantitative risk assessment; important issues to remember are the need for high quality data, extensive validation of models in foods, and avoiding extrapolation beyond the conditions used to generate the model (McNab, 1997).

In QRA, mathematical models are used to estimate the ultimate risk to the consumer as a function of input values taken from various points along the ‘farm-to-fork’ continuum. Due to heterogeneity of microorganisms, variability around single point estimates of risk can be significant. Thus, point estimates give limited information, describing single instances such as ‘worst case’ scenarios (Buchanan and Whiting, 1996; Lammerding and Fazil, 2000). Improvements in prediction can be made by incorporating uncertainty. Uncertainty is an important factor in risk analysis, since it limits our ability to make reliable predictions of risk. Uncertainty may arise from inherent variability in the biological system, or from lack of information or understanding of the mechanisms involved (McNab, 1997). Uncertainty can be minimised by obtaining more, high quality data; however, as this is not always feasible, alternatives must be sought. One approach is to describe variability using probability distributions to represent parameter values. These distributions can be built from empirical data, knowledge of underlying biological phenomena, or expert opinion (Lammerding and Fazil, 2000), and the process leads to an output where risk is expressed as a probability distribution. Risk analysis software such as @RISK™, which uses Monte Carlo analysis to simulate output distributions of risk based on variability of input data, can facilitate the risk assessment process (Buchanan and Whiting, 1996; Lammerding and Fazil, 2000).

Nauta (2000) has emphasised the need to separate true biological variability due to heterogeneity of populations from uncertainty, the lack of perfect knowledge of the parameter values. This is commonly neglected in risk assessment studies. Working with data on growth of *Bacillus cereus* in pasteurised milk, Nauta (2000) showed that prediction of outbreak size may depend on the way that uncertainty and variability are separated.

The extended application of risk assessment procedures to the survival of *E. coli* O157:H7 in ground beef hamburgers was studied by Cassin *et al.* (1998)

who defined the term Process Risk Model to predict the probability of an adverse impact as a function of multiple process parameters. This approach is based on the assumption that risk is determined by process variables, and that behaviour of the microorganism can be described mathematically. Simulations were done with the Monte Carlo approach, and rank correlations were used to find variables which were most strongly correlated with illness. This process allowed the identification of CCPs.

6.6 Risk assessment and pasteurisation

Mathematical modelling has been applied to dairy products and processes (Griffiths, 1994); however, there has been limited application of QRA. Risk assessments have been done for the growth of *B. cereus* in pasteurised milk (Zwietering *et al.*, 1996; Notermans *et al.*, 1997), survival of *L. monocytogenes* in soft cheese (Bemrah *et al.*, 1998), and survival of *L. monocytogenes* during milk processing (Peeler and Bunning, 1994). This latter study also incorporated estimates of survival with pasteurisation at several time–temperature combinations. Some of the limited number of risk assessments performed have been reviewed, and a number of weaknesses and omissions have been identified (Cassin *et al.*, 1996; Schlundt, 2000). The process of QRA is still in its infancy, however, and standards have yet to be developed. There is a clear advantage to the food industry and consumers to further develop the concepts of QRA and apply them to both common and novel food processes, and it is expected that significant advances will be made in this field over the next decade.

Returning now to the pasteurisation models, we note that mean parameter values (Table 6.2) provide single output values for each set of processing conditions, but do not take into account inter-trial variability. In order to make these models more relevant to QRA, the risk analysis software @RISK™, a Microsoft Excel add-in, was used. @RISK™ expresses model parameters as distributions, and when simulations are performed, outputs are calculated as distributions. Thus, a range of probable output (e.g. survival) values for a specified set of processing conditions is obtained, and the probability of achieving a target log reduction can be estimated.

An Excel spreadsheet was prepared which contained the model and calculations of PE and \log_{10} reduction for each of the target enzymes or pathogens. Model parameters (E_a/R , intercept, slope and power) were entered into the spreadsheet as normal distributions with mean and standard deviation values taken from previously published information for each target (Table 6.2). For some targets, it was discovered that parameter estimates were correlated. For example, significant correlations were observed between E_a/R and both slope ($r^2 = 0.647$) and intercept ($r^2 = 0.240$) for the five *L. innocua* trials. During simulations, @RISK™ normally takes random sample values from each of the input distributions. If parameter values are correlated, it is necessary to have @RISK™ adjust sampling patterns to include these correlations.

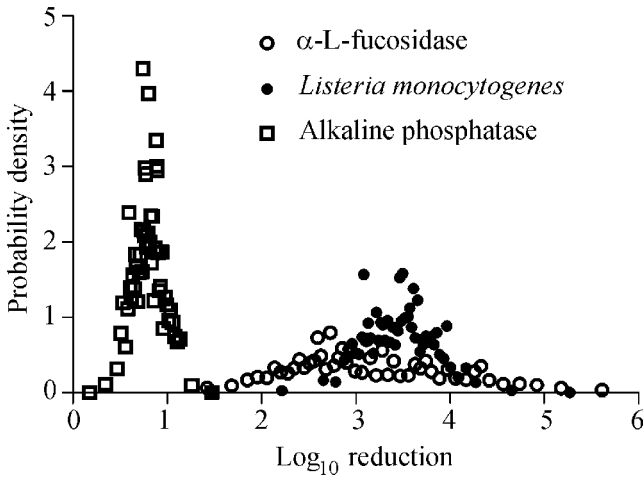


Fig. 6.8 Probability distributions for α -L-fucosidase, *Listeria monocytogenes*, and alkaline phosphatase generated from @RISK™ simulations with processing at 65°C for 15 s.

Monte Carlo is the traditional method for sampling distributions in which samples are taken randomly throughout the input distribution (Vose, 1996). Latin hypercube is a recent development in sampling technology which employs stratification of the input probability distributions, resulting in fewer iterations than the traditional Monte Carlo method. For these studies, Latin hypercube simulations were done with a total of 1500 iterations for each replicate simulation. As an example of this process, simulated log reductions were generated for AP, FC and *L. monocytogenes* using a holding time of 65°C for 15 s (corresponding to thermisation), and the probability density functions are shown in Fig. 6.8. These conditions resulted in a narrow band of probabilities for AP, with greater predicted range for both FC and *L. monocytogenes*. Thermisation does not completely inactivate AP, while FC (a potential indicator of thermisation) experiences a greater than 2 log reduction in most iterations. The mean log reduction of *L. monocytogenes* under these conditions is greater than 3.

Improvements in predictive power from @RISK™ models can be realised by the use of percentiles. Probability distributions generated by simulation are divided into equal probability increments called percentiles. Percentiles represent the percentage of generated results which are less than or equal to the associated log reduction. A failure is scored when the predicted inactivation is greater than the experimental value at that percentile (i.e., a 'fail-dangerous' prediction). The lower percentiles are associated with lower, more conservative estimates of log reduction at a particular set of processing conditions.

As an example, the results of simulations carried out with three *E. sakazakii* trials are shown in Table 6.3 (Nazarowec-White *et al.*, 1999). At the 5th percentile, the model 'failed dangerous' with Trials A and B; however, the more

Table 6.3 Validation of *E. sakazakii* model using @RISK™ simulations^a

Percentile ^b	Number of failures ^c								
	Trial A (n = 12)			Trial B (n = 24)			Trial C (n = 22)		
	1 ^d	2	3	1	2	3	1	2	3
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	1	1	0	0	0
5	1	1	1	3	3	3	0	0	0
10	2	2	2	8	8	8	4	4	3
15	3	3	3	12	12	13	12	12	11
20	3	3	3	16	16	16	15	15	15
25	4	4	4	19	19	18	17	17	18
30	6	6	6	20	19	19	18	18	18
35	7	7	7	20	20	20	18	18	18
40	8	8	8	20	20	20	19	19	19

^a Data are from Nazarowec-White *et al.* (1999) reprinted with permission from *Food Research International*. Copyright held by Elsevier Science Publishers.

^b Probability distributions generated by simulation are divided into equal probability increments called percentiles. Percentiles represent the percentage of simulated results which are less than or equal to the associated log reduction.

^c A failure is scored when the predicted inactivation was greater than the experimental value at that percentile (i.e., a 'fail-dangerous' situation). The lower percentiles are associated with lower, more conservative, estimates of log reduction at a particular set of processing conditions.

^d Replicate simulations.

conservative estimated log reduction associated with the 1st percentile did not result in any failures (Table 6.3). At the higher percentiles, a greater number of failures are observed, indicating that the corresponding predicted log reductions are too ambitious, and unlikely to be achieved in practice. This approach allows the user to set the level of allowable risk, then select the processing conditions which will result in the desired degree of inactivation. The value of this approach was further examined by comparing thermal inactivation of *E. sakazakii* with that of *L. monocytogenes*. Results of the comparison (Table 6.4) show that at all temperatures simulated, *E. sakazakii* was more heat-sensitive than *L. monocytogenes*, with greater than 1-log difference at 68°C (Table 6.4). Comparison of simulated log₁₀ reductions associated with the 1st and 5th percentiles revealed that *E. sakazakii* was only slightly more heat-sensitive at 68°C than *L. monocytogenes*, with differences of 0.5 and 0.25 log at the two percentiles, respectively (Table 6.4). This apparent decreased difference in heat sensitivity between the two pathogens associated with the 1st and 5th percentile predictions reflects the greater uncertainty in the slope parameter for the *E. sakazakii* model compared to that for the *L. monocytogenes* model (Nazarowec-White *et al.*, 1999).

Models which can predict the probability of achieving a desired level of safety are an important addition to risk assessment models which are still largely

Table 6.4 Comparison of log₁₀ reductions for *Enterobacter sakazakii* and *Listeria monocytogenes* simulated using @RISKTM^a

Temperature (°C)	Simulated log ₁₀ reduction ^b					
	<i>Enterobacter sakazakii</i>			<i>Listeria monocytogenes</i>		
	Mean (S.D.)	Percentile ^c		Mean (S.D.)	Percentile ^c	
		5	1		5	1
67.0	5.46 (0.626)	4.16	3.65	4.88 (0.472)	3.89	3.47
67.5	6.51 (0.728)	5.04	4.43	5.71 (0.484)	4.67	4.23
68.0	7.75 (0.850)	6.08	5.36	6.69 (0.500)	5.59	5.13

^a Data are from Nazarowec-White *et al.* (1999) reprinted with permission from *Food Research International*. Copyright held by Elsevier Science Publishers.

^b 16 s holding time; 1500 iterations.

^c Percent of the total iterations which give simulated log₁₀ reductions less than the values corresponding to each temperature. For example, at 67.0°C, the *E. sakazakii* model predicted a mean log₁₀ reduction of 5.46, with 5% of the 1500 iterations giving a value of <4.16. Thus, in 95% of the simulations, the model predicts >4.16 log₁₀ reduction.

qualitative, and based primarily on expert opinion. To facilitate this process, the pasteurisation models described above have been incorporated into the risk analysis software, Analytica[®], which is commonly used for building risk assessment models for the food industry. This software supports a modular approach, thus pasteurisation models may be easily incorporated into larger risk assessment models designed to encompass the entire ‘farm-to-fork’ continuum. Models for microbial survival and growth have also been incorporated into several large databases which are available to the food industry. Food MicroModel, which is based on work done by the Institute of Food Research in the UK and their collaborators (McClure *et al.*, 1994), is a commercially available software package which is continually updated, but which requires an annual licensing fee. The Pathogen Modelling Program (PMP) was designed by the United States Department of Agriculture to provide assistance to the food industry, and is available free of charge (Buchanan, 1993). Pasteurisation models discussed here will be incorporated into the PMP.

6.7 Future trends

The models described in this chapter have thus far been confined to enzymes and pathogens in whole milk. Other dairy products are also of concern, thus models will continue to be expanded to include more viscous dairy products such as ice-cream mix. For example, preliminary studies have been undertaken to assess the extent to which the thickening agent guar gum can influence HTST processing (Piyasena and McKellar, 1999). Other liquid food and beverage

products may also be potential sources of risks. Apple cider is often sold unpasteurised to consumers who consider this product to be more natural; however, *E. coli* O157:H7 is often a potential risk in this product. Limited thermal treatment of this product, combined with other intervention strategies, may present a solution. To this end, preliminary HTST models have been developed for a model microorganism, *Pediococcus* sp., in simulated apple cider (Piyasena *et al.*, 2003).

Other thermal and non-thermal processing technologies are gaining in popularity. Current regulations which require specified time–temperature treatments are based on thermal processes, thus it is difficult to establish equivalent process conditions. Some of these technologies, such as radio frequency (RF) and ohmic heating, are thermal processes, and thus may be modelled by some modification of the present HTST models. In contrast, the mechanisms by which non-thermal processes such as pulse electric field (PEF) and ultraviolet light inactivate microorganisms are as yet poorly understood. It has become apparent that further work is needed to establish adequate modelling approaches for these technologies (Institute of Food Technologists, 2000).

It is now well known that thermal resistance of bacteria can be influenced by the conditions or stage of growth. Production of heat-shock proteins by bacteria renders them increasingly resistant to thermal treatment. Cross-protection with other stresses such as low pH and starvation has also been observed. Development of adequate models to describe these phenomena will require a greater knowledge of the physiological changes taking place in bacterial cells, thus there will be a move away from empirical modelling to more mechanistic models which are based on expression of key genes or synthesis of heat-shock proteins essential for survival. This molecular modelling approach is one which is being actively explored worldwide. Our research group is closely involved with a large molecular modelling project with the University of Guelph.

It is well known that bacterial survival curves are rarely first-order, yet no concerted approach to this problem has been suggested. The use of distributions, which imply heterogeneity of cells, seems like a worthwhile and most promising approach. Recent interest in the heterogeneity of bacterial cells (Booth, 2002) and an improved understanding of the factors which determine intra-cell variations in heat resistance will further strengthen our ability to provide adequate models to the food industry.

6.8 Sources of further information and advice

Important references include McMeekin *et al.* (1993), a reference book describing some of the principles of predictive food microbiology; Vose (1996), a book describing the fundamentals of risk analysis and Monte Carlo simulation; and more recently a book on food process modelling with specific chapters on modelling uncertainty (Van Impe *et al.*, 2001), food safety (Baranyi and Pin, 2001) and thermal processing (Nicolai *et al.*, 2001 and Bakalis *et al.*, 2001).

The PMP is available from the USDA at: <http://www.arserrc.gov/mfs/pathogen.htm>

Food MicroModel is available from Leatherhead Food RA at: <http://www.lfra.co.uk/>

Other useful sites:

IDF: <http://www.fil-idf.org/>

National Milk Producers' Federation (US): <http://www.nmpf.org/>

National Dairy Council (US): <http://www.nationaldairycouncil.org/>

National Dairy Council of Canada: <http://www.ndcc.ca>

Agriculture and Agri-Food Canada: http://www.agr.gc.ca/index_e.phtml

Canadian Dairy regulations: <http://www.dairyinfo.agr.ca/cdicdrca.htm>

United States Dairy regulations: <http://www.dairyinfo.agr.ca/cdicdrusa.htm>

International Dairy regulations: <http://www.dairyinfo.agr.ca/cdicdrint.htm>

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