

5 Challenge of Food and the Environment

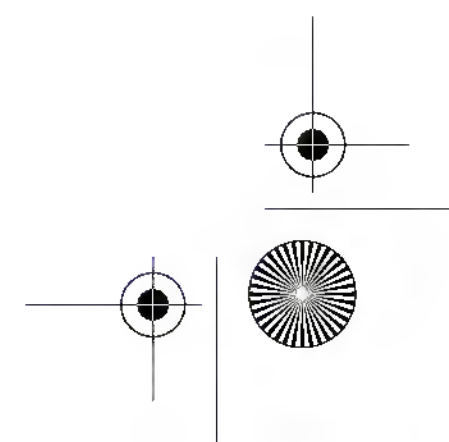
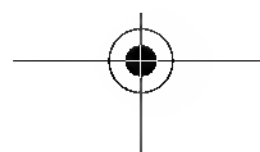
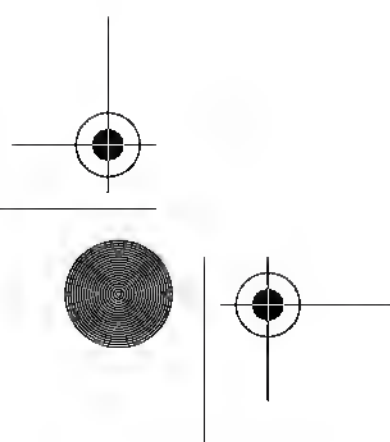
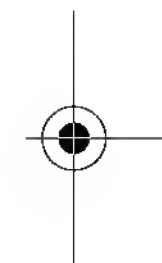
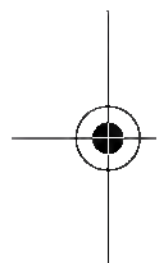
Tim Brocklehurst

CONTENTS

- 5.1 Role of Food Heterogeneity
 - 5.1.1 Aqueous Phase
 - 5.1.2 Gelled Aqueous Phase
 - 5.1.3 Oil-in-Water Emulsions
 - 5.1.4 Water-in-Oil Emulsions
 - 5.1.5 Gelled Emulsions
 - 5.1.6 Surfaces
 - 5.2 Modeling the Food Environment
 - 5.2.1 Organic Acids
 - 5.2.2 Dissociation
 - 5.2.3 Partitioning into Oil Phases
 - 5.2.4 Water Activity
 - 5.3 Hurdle Concept
 - 5.4 Competition with Other Microorganisms
 - 5.4.1 Interactions Based on the End-Products of Metabolism of One Species
 - 5.4.2 Mixed Culture
 - 5.5 Adaptation and Injury
 - 5.5.1 Effects of Environment on Adaptation
 - 5.5.2 Effects of Sublethal Injury
 - 5.5.2.1 Enumeration of Sublethally Injured Bacteria
 - 5.6 Validation in Foods
 - 5.6.1 Bias and Accuracy
 - 5.6.2 Validation Using Literature Values
 - 5.6.3 Validation in Foods
- References

5.1 ROLE OF FOOD HETEROGENEITY

Foods are typically not homogeneous. The structure of the food creates local chemical or physical environments that affect the spatial distribution of microorganisms



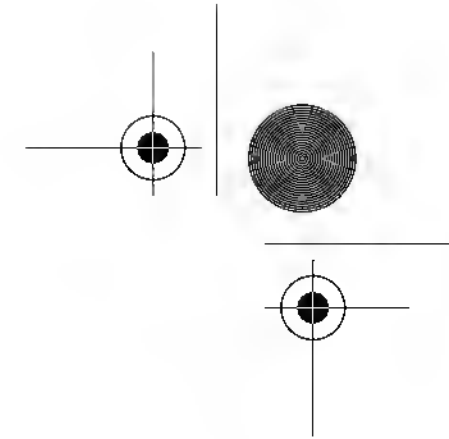
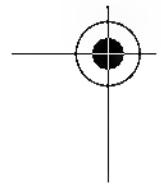
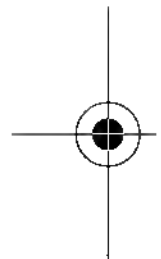


TABLE 5.1
Examples of the Heterogeneity of Foods

Structure of the Food	Examples of Food	Model Experimental Systems Used to Mimic This Food Structure
Liquid	Soups, juices (with some suspended material)	Broth culture medium
Gel	Pate, jellies, skimmed milk cheeses, such as cottage cheese	Cells immobilized in agar or gelatin (including in a specifically designed Gel Cassette System)
Oil-in-water emulsion	Dairy cream, milk, salad cream, mayonnaise	Alkane:culture medium emulsions
Water-in-oil emulsion	Butter, margarine, low fat spread	Culture medium:alkane emulsions
Gelled emulsion	Whole milk cheese	Alkane:culture medium emulsions, where the aqueous phase is gelled with agarose
Surface	Vegetable tissues, meat tissues	Agar or gelatin (including a modified version of the Gel Cassette System)

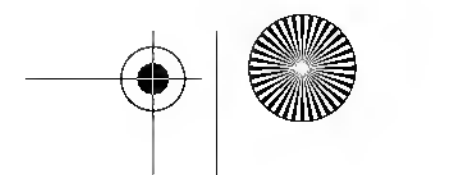
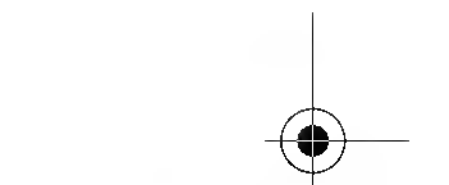
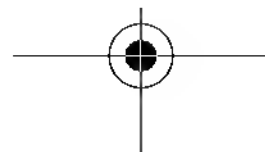
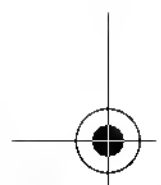


as well as their survival and growth.¹⁹⁷ Microorganisms occupy the aqueous phase of foods, and structural features of this phase (Table 5.1) relevant to the length scale of microorganisms can influence their growth. The effects of these structural features on microbial growth include constraints on the mechanical distribution of water,^{77,78} the redistribution of organic acids, including those used as food preservatives,^{31,32} and constraints on the mobility of microorganisms.^{30,60,61,109,110,139,152,153,201}

Many foods will contain a number of microstructural features, and the behavior of microorganisms is influenced differently in each. For example, Parker et al.¹⁴⁰ described the effect of microstructure on the distribution and growth of microorganisms in Serra cheese. Some growth occurred in liquid regions, while other microorganisms formed colonies on surfaces and within the protein gel of the curd (Figure 5.1). Predictions based on data obtained from broth systems can be applied successfully to organisms growing in structured foods. However, where the structure of the food results in a different behavior, this is described below, together with model experimental systems for its study. In many cases growth is “fail-safe,” in that organisms grow more slowly in structured systems than in broths. Wilson et al.¹⁹⁷ suggested that this may explain the differences that food manufacturers sometimes observe, where challenge testing of real foods indicates growth at a slower rate than suggested from predictive models. Additionally, the complexity of food structure has been identified as a major contribution to the “overall error” included in microbiological modeling predictions.¹⁴⁴

5.1.1 AQUEOUS PHASE

Growth in a liquid aqueous phase is typically planktonic, with motility allowing taxis to preferred regions of the food. Diffusive transport of nutrients to



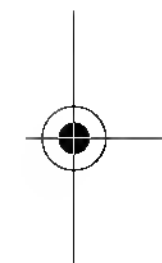
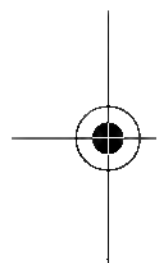
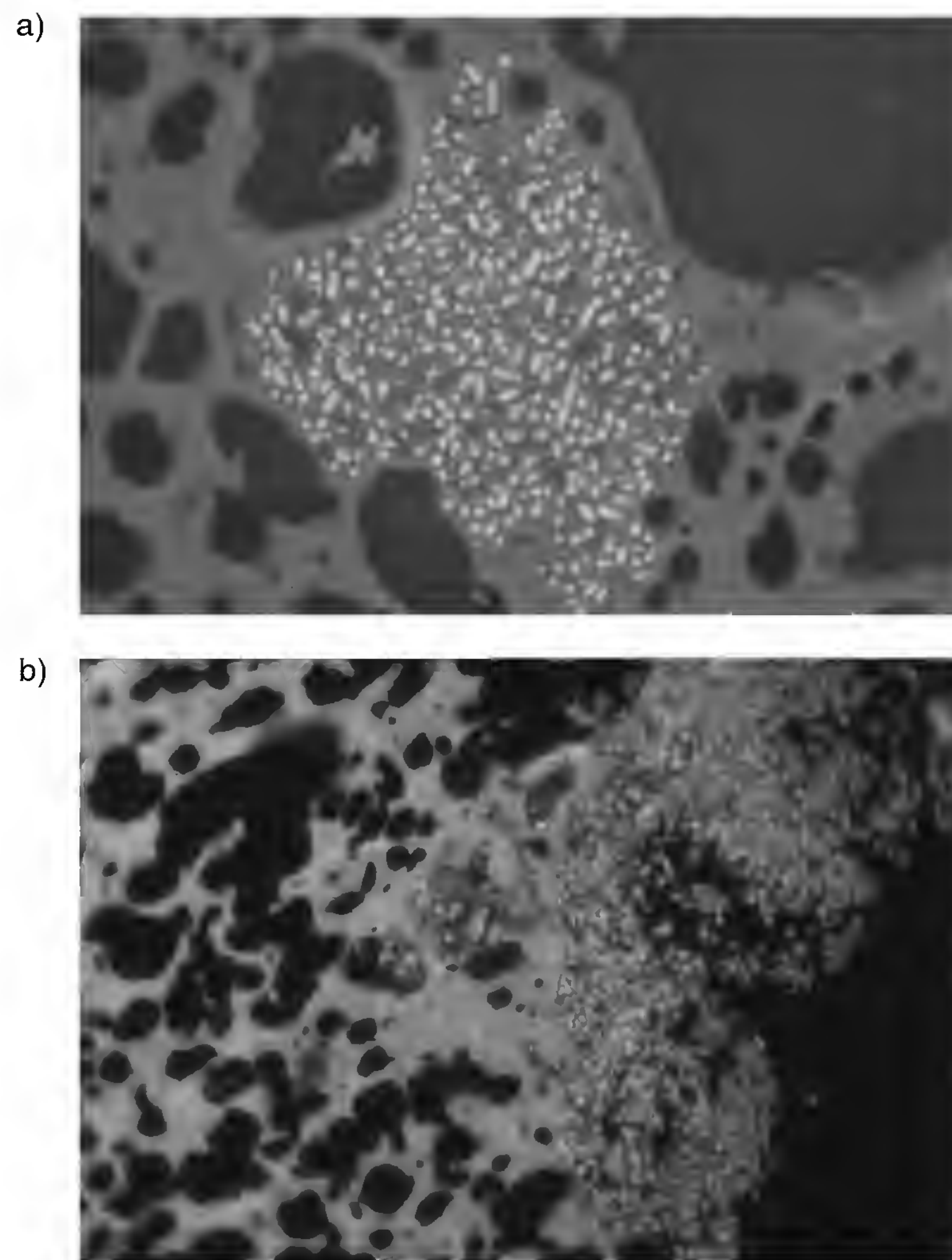
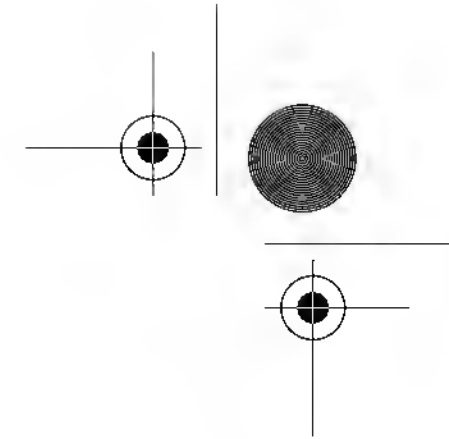
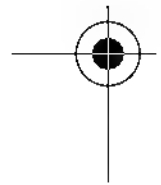
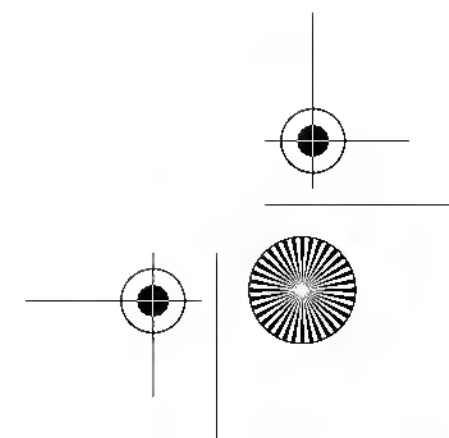
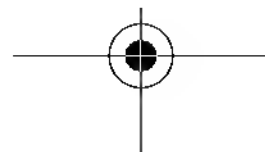
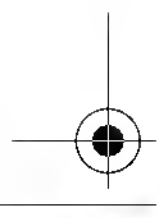
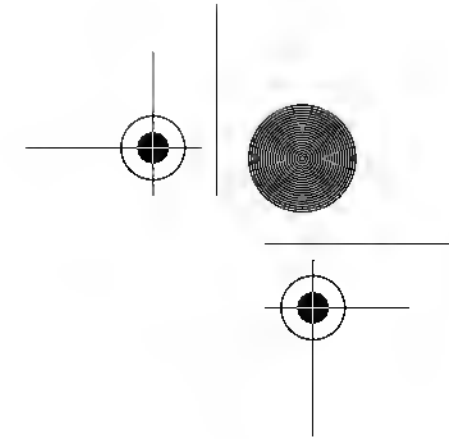
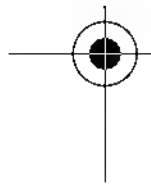


FIGURE 5.1 Light micrographs demonstrating some structural heterogeneity in hard cheese, and showing (a) a colony embedded within the gelled protein of the cheese curd and (b) a colony growing on the surface. The black irregular shapes are embedded globules of milk fat.

microorganisms and of their metabolites away can result in a locally stable equilibrium environment until accumulation of microbial biomass and metabolites cause bulk chemical changes. This is typically manifested by changes in pH or in gaseous composition. When broth culture medium is used in microbiological experiments it is this environment that is mimicked, and, with few exceptions, models for bacterial growth and death have been developed in such simple broth systems. The complexity of foods has been recognized for many years, and it has been suggested that the development of detailed models to account for all aspects of microbial growth in foods may be too costly, and will not yield useful





intermediate models.¹⁴ Simplifying assumptions can be made, and models derived in this way have proved useful.¹⁴

However, with improving knowledge and the advent of mechanistic modeling approaches it is possible to make predictions of the behavior of microorganisms in structured foods.

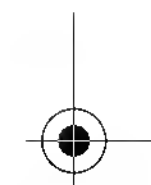
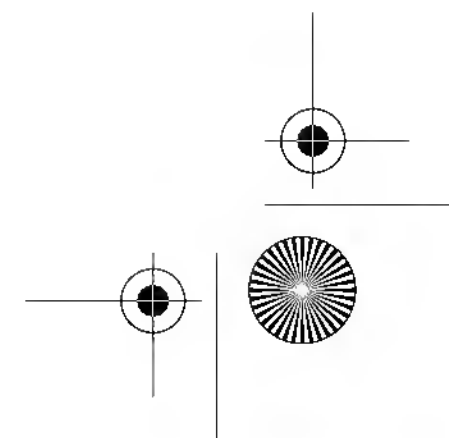
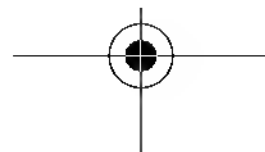
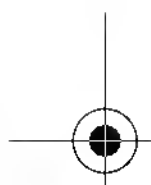
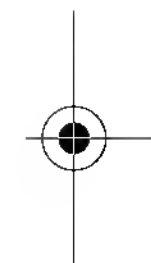
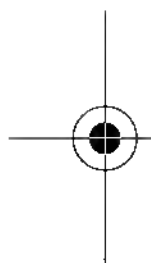
5.1.2 GELLED AQUEOUS PHASE

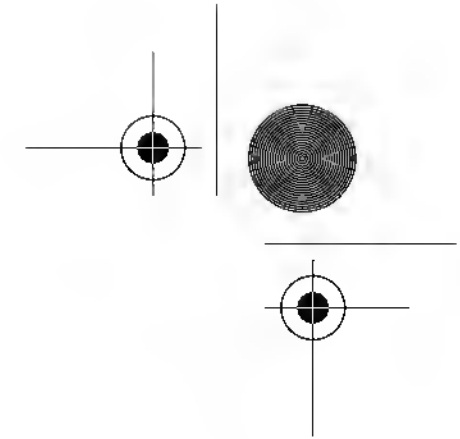
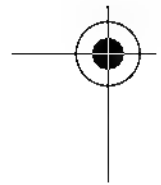
In gelled regions microorganisms are immobilized. This can occur as single isolated cells, or when these multiply, they are constrained to grow as colonies.^{60,61,84,88,140,201}

Model experimental systems for studying colonial growth include agar^{17,124} and gelatin in a specifically designed Gel Cassette System.²⁸ Immobilized growth as colonies results in local depletion of oxygen^{200,201} and local accumulation of end-products of metabolism, which results in a local decrease in pH within and around the colonies.^{104,192,201} Immobilized bacteria also differ from planktonic cultures in their susceptibility to antimicrobial compounds, their energy metabolism, and their metabolic end-products.^{165,193} Accordingly, in gelled regions of foods, the growth of microorganisms will result in local changes in the concentration of their growth requirements and metabolites. This results in growth at a slower rate and to a lower yield than planktonic, or free-living cells.^{30,152} A unifying theory of microbial growth, which includes proposed equations for a structured-cell mathematical model, influences of local environmental conditions on growth, influences of the microorganisms themselves on the environment, transport of solutes between phases, and physical expansion of colonies,¹⁵² has been developed to attempt explanation of these growth characteristics.⁷⁹ Experimental data demonstrate both a decrease in growth rate and shrinkage of habitat domain in the case of *Listeria monocytogenes*, *Listeria innocua*, and *Bacillus cereus*. In all of these cases, the use of a predictive model based on data from the broth experiments would lead to a “fail-safe” prediction in the gelled system. However, Wilson et al.¹⁹⁷ described the growth of *Staphylococcus aureus* as a function of sucrose concentration. In the absence of sucrose, growth was slower than in the broth cultures when the cells were immobilized in gel. However, as the concentration of sucrose was increased, the growth rate in broth decreased, but remained unaffected in gel. Hence, these authors identified conditions of a concentration of sucrose above ca. 15% (w/v) at pH 6 where growth was faster in the case of cells immobilized in gel than for cells in broth (i.e., “fail-dangerous” if a model prediction was based on data from broth cultures).

Growth of cells immobilized in gelatin has been examined under nonisothermal conditions.²⁸ This study showed that immobilized cells differ from planktonic bacteria during temperature cycling when stressed by high salt or low pH. A finite-difference scheme has been used to combine thermal inactivation modeling with thermal conduction modeling to simulate inactivation of bacteria immobilized within agar blocks.¹⁷

The local accumulation of metabolic end-products within and around colonies can result in interaction between them. Such competition resulting from close spatial distribution has been termed propinquity, and occurs up to a separation distance of between 1400 and 2000 μm .^{177,201} The authors of these works go on to emphasize



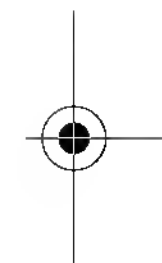
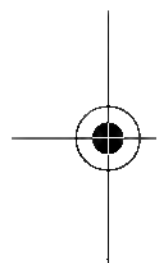


that a gap exists between model systems and food, and that to bridge it requires the combined efforts of food microbiologists and microbial physiologists.²⁰¹

5.1.3 OIL-IN-WATER EMULSIONS

Here, structure is affected by the concentration and form of the oil phase. The concentration of oil in food varies considerably,³² and in milk is typically between 3 and 5% (v/v), but in mayonnaise may be between 26 and 85% (v/v). The oil phase exists as polydispersed droplets with a mean diameter that is typically between 0.15 and 8 μm . In concentrated emulsions, the space of the interstices between the droplets is of the same order of size, which is also the same order of size as many bacteria.

In model experimental systems a relationship exists between the concentration of oil and the form of growth of microorganisms.¹³⁹ Where the concentration of lipid phase was low (30% v/v) the growth of bacteria was as free-living (or planktonic) cells. An increase in the concentration of the oil phase had no effect on the form of growth of bacteria until it was increased to 83% (v/v). Here the bacteria became immobilized between the close-packed oil droplets. This entrapment resulted in growth not as planktonic cells, but as discrete colonies. The droplets within emulsions confer opacity, and hence visualization of microorganisms is difficult. A mixture of chloroform and methanol was used to selectively remove the oil phase and allow the examination of colonies *in situ*.^{30,139} The investigators showed that the colonies are formed from a single bacterium, and as they expanded they displaced the emulsion droplets. Immobilization of bacteria by the lipid component and subsequent growth as colonies resulted in a decreased rate of growth and a shrinkage of the habitat domain compared with growth as planktonic cells — essentially, similar results to the consequences of colonial growth in gels.

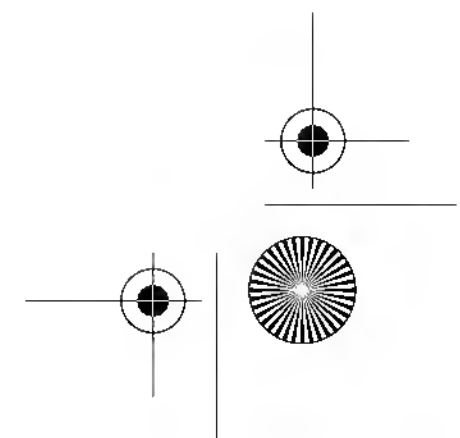
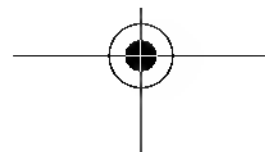
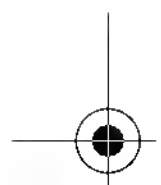


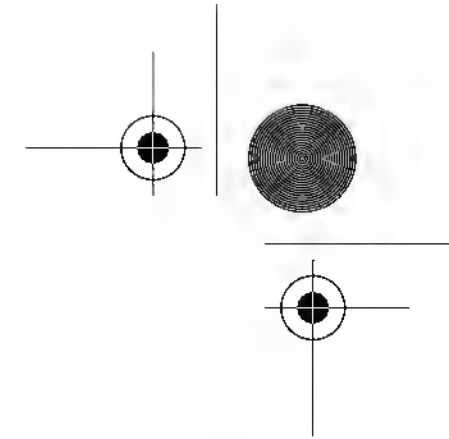
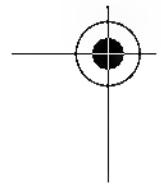
5.1.4 WATER-IN-OIL EMULSIONS

These consist of an internal aqueous phase dispersed as discrete spherical or irregularly shaped droplets within an outer oil phase, which may contain a mixture of fluid and crystalline fats. In the case of margarines the droplets of aqueous phase are typically irregular in shape, and can range between 0.3 and 30 μm in diameter.¹⁸⁶

Droplets can be contaminated with microorganisms at the point of emulsion manufacture.¹⁸⁶ The proportion of droplets occupied by microorganisms is small, and a model to predict microbiological contamination based on a function of the initial contamination, and the numbers of droplets exceeding the minimum size for occupancy, has been developed.¹⁸⁶

Classical theories to describe microbial growth rely on the maintenance of discrete compartmentalized droplets that restrict the availability of water, space, and nutrients for growth. On the basis of these assumptions, Verrips and Zaalberg¹⁸⁶ and Verrips et al.¹⁸⁷ used a mechanistic approach to predict the growth of bacteria within discrete droplets related to the dimensions of the occupied droplets. This was expanded further by modeling the energy demands of the contained bacteria.¹⁷⁵ Models are useful here to predict states that are difficult to measure, and predictions confirm that bacteria in the droplets can grow well, but that their numbers remain





small when expressed per unit volume of emulsion (although their local number density within a droplet is extremely high). Additionally, microorganisms cease to grow when the concentration of metabolic end-products (typically organic acids) becomes toxic or if a requirement for growth, such as oxygen or a carbon source, is exhausted. Models confirm that bacterial growth is restricted when the food structure remains intact (i.e., when coalescence of the droplets does not occur). This was observed in model experimental systems where an increase in numbers of bacteria in water-in-oil emulsions was always accompanied by coalescence of the droplets of aqueous phase.³¹

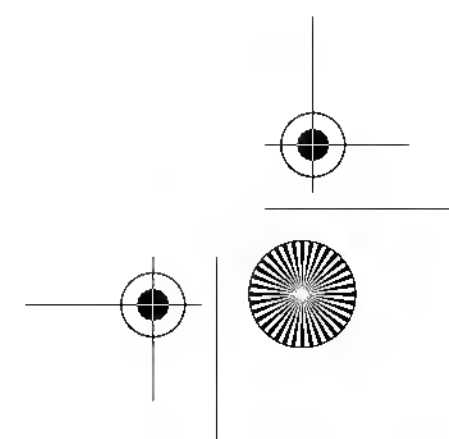
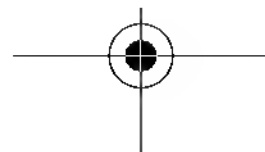
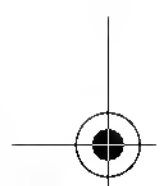
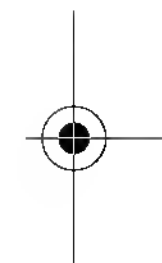
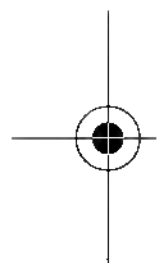
5.1.5 GELLED EMULSIONS

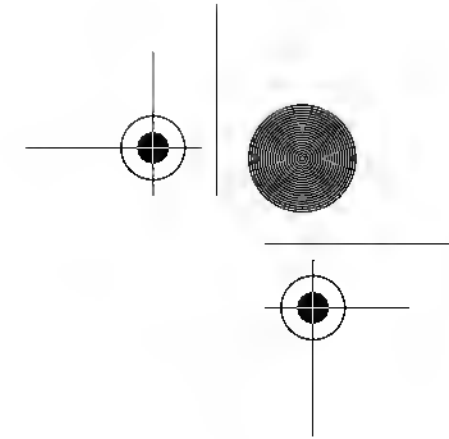
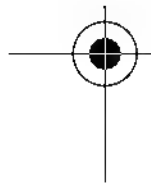
Many food emulsions are gelled. This can occur by the deliberate addition of gums or thickeners to increase the bulk viscosity (such as in sausages) or the denaturation of protein to form protein micelles (such as in cheese). Microorganisms are immobilized and constrained to form colonies much as in gelled systems described above.^{60,61,140}

5.1.6 SURFACES

The simplest form of food structure is the surface. Growth of bacteria on the surface of food has been measured on Canadian wieners,¹¹⁸ pâté,⁶⁹ and vegetable tissues.²⁷ Model experimental systems are numerous and include agar gels,^{53,115,168,179,199,202} agar film,¹¹⁵ two-dimensional gradient plates,^{178–180,203,204} and a modification of the Gel Cassette mentioned above.²⁹

Nicolai et al.¹³² modeled surface growth with the assumption that it was in a surface film of liquid. However, growth on a surface is typically colonial. Hence, constraints on growth are similar to those described in the case of gels. Some key differences are important in modeling. Crucially, diffusion limitations are greater at a surface than within an enveloping gel. This was confirmed by Wimpenny and Coombs,²⁰⁰ Peters et al.,¹⁴¹ and Robinson et al.¹⁵⁵ who measured the depletion of oxygen and accumulation of protons immediately beneath the colony and extending into the substratum. Colonial growth on surfaces results in decreased growth rates, and comparisons of the growth rates of *Salmonella typhimurium* affected by increasing salt or sucrose followed the order: broth > immersed colonies > surface colonies.²⁹ This suggests that the rate of growth on surfaces may not be well predicted by models derived from broth systems.²⁹ Spatial distribution on a surface leads to interactions between colonies.¹⁷⁶ Spatial and temporal variations have a major influence on the potential of surfaces to support bacterial growth. In foods, it is particularly the availability of water.⁵⁰ Drying of a food may be deliberate to inhibit growth, and desiccation of microorganisms has been reviewed.¹⁴⁶ A solid surface model system was developed to study the effect of gas atmosphere on growth of several psychrotrophic pathogens.²¹ This system demonstrated that increased CO₂ markedly inhibited the growth of all pathogens. The model system can be applied to examination of the growth of pathogens on minimally processed produce under modified





atmospheres. Radial growth of colonies of *B. cereus* on a solid agar surface was dependent on interaction between agar concentration and water activity.¹⁶⁸

5.2 MODELING THE FOOD ENVIRONMENT

In order to predict the growth of microorganisms in foods reliably, it is vital to use the correct initial chemical conditions. The structural heterogeneity of foods results in a chemical heterogeneity, which is often complicated by dynamics within the food that create a “new” chemical environment. Models of varying complexity exist that can predict the true initial chemical state of foods. Microorganisms occupy the aqueous phase of foods,^{30,184} and hence, it is the chemical composition of this phase that requires accurate prediction.

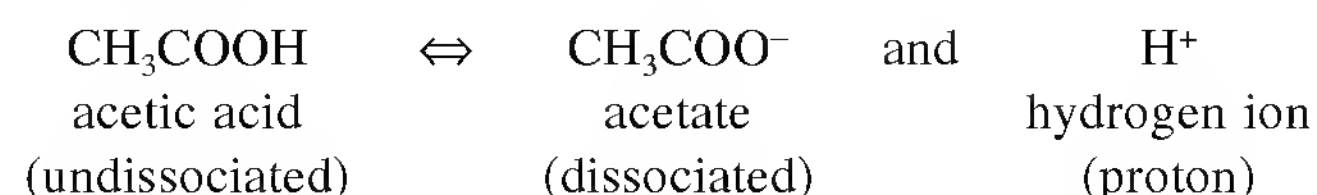
Many foods rely for their preservation on the concentration of organic acids (e.g., acetic, lactic, benzoic, or sorbic acid). In addition, the concentration of sugars or salts can contribute to preservation. It is, therefore, no surprise that many predictive models use combinations of pH and water activity (although often expressed as concentration of NaCl) together with temperature as the three major determinants of growth. What follows is a summary of available models that can predict the initial environmental conditions within foods.

5.2.1 ORGANIC ACIDS

Acetic, lactic, benzoic, and sorbic acid (and their salts) are added as preservatives in many foods, although acetic and lactic acids are also produced in fermented foods as end-products of microbial metabolism. Their preservative action is by virtue of a combination of their effect on the pH of the food and the antimicrobial properties of the undissociated form of the molecule. Accordingly, their antimicrobial effect is influenced by the fundamental thermodynamic characteristics of dissociation and partition. It is these that must be modeled to predict the potential of foods to inhibit the growth of microorganisms.

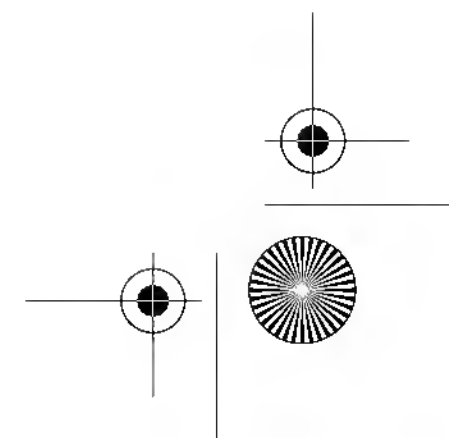
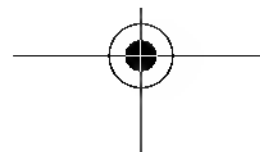
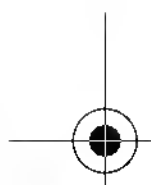
5.2.2 DISSOCIATION

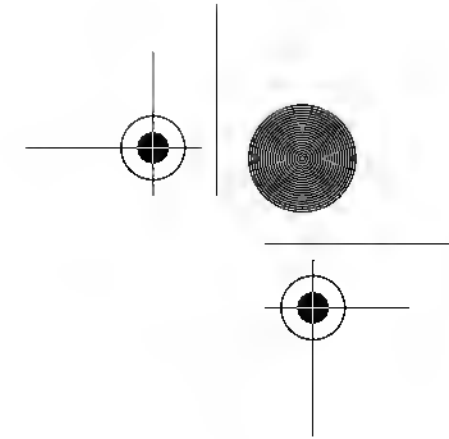
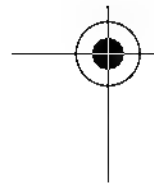
Weak organic acids dissociate (or separate) into their component parts. In the case of acetic acid, this occurs as:



This dissociation is key to prediction of the concentration of the undissociated form of the acid, which has the predominant antimicrobial effect in foods.^{11,65,166}

The Henderson–Hasselbalch equation relates the pH of the food to the pK_a and the relative proportions of dissociated and undissociated acid in foods have been predicted¹⁹⁸ as follows:





$$pH = pK + \log_{10} \frac{[acid]_{dissociated}}{[acid]_{undissociated}} \quad (5.1)$$

Rearrangement gives the concentration of weak acid in its undissociated (i.e., micro-biologically active) form, $[HA]_{aq}$, given the pH, pK_a , and total concentration of weak acid, $[HA]_T$, as follows:

$$[HA]_{aq} = \frac{[HA]_T}{1 + 10^{(pH - pK_a)}} \quad (5.2)$$

where $[HA]_{aq}$ is the concentration of undissociated organic acid in the aqueous phase and $[HA]_T$ is the total concentration of organic acid. pK_a is the negative logarithm of the dissociation constant K_a , which is a thermodynamic constant controlling the dissociation equilibrium shown above:

$$pK_a = -\log(K_a) \quad (5.3)$$

K_a is typically a small number, and published values are available.²⁰⁵ pK_a varies slightly with temperature, and an empirical equation that predicts this variation has been published¹⁵⁴:

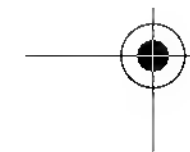
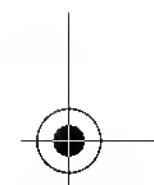
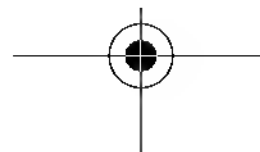
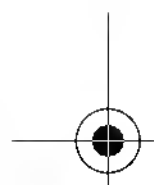
$$pK_a = \left(\frac{A}{T} \right) - B + (CT) \quad (5.4)$$

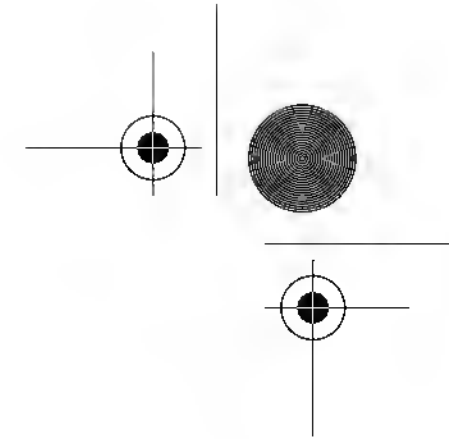
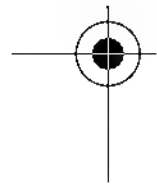
where T is the temperature in Kelvin (K), and A , B , and C are shown in Table 5.2. Such predictions are important preliminaries in dealing with the challenge of food and the environment. Without such knowledge it is quite simple to apply an incorrect initial environmental condition when using predictive microbiology tools, and this can easily result in erroneous predictions.

Predictions must also be reiterative. For example, once dissolved, the organic acid will dissociate depending upon local pH, but will then perturb this pH. The dissociation is also dependent on local buffering capacity of the food, and this is

TABLE 5.2
Values of A , B , and C to Be Inserted into
Equation 5.4 for Calculation of the Effect
of Temperature on pK_a ¹⁵⁴

Acid	A	B	C
Acetic	1170.48	3.1649	0.013399
Lactic	1286.49	4.8607	0.014776
Benzoic	1590.2	6.394	0.01765





extremely difficult to predict. However, Wilson et al.¹⁹⁸ developed a method for performing calculations describing the reiterative dissociation of organic acids, and hence predicting the true chemical composition of foods. This not only allows microbial growth models to predict growth, but also allows the changes in pH caused by microbial metabolism to be predicted. These authors used a theory describing the behavior of weakly dissociating systems, and knowledge of dissociation constants and concentrations. They make the point that food is too complex for solutions to be achieved through complex calculation. Hence, the authors characterized the buffering behavior of food by a titration with a strong (i.e., completely dissociating) acid, and then used knowledge of the dissociation constants of weak acid preservatives to predict the behavior of these in the food. Their calculation scheme may also be applied to a mixture of weak acids including polyacid species such as the tricarboxylic acids (e.g., citric acid).¹⁹⁸

5.2.3 PARTITIONING INTO OIL PHASES

In biphasic foods, which contain aqueous and lipid phases, the antimicrobial undissociated acids partition between the aqueous and lipid components.³² This decreases the concentration of undissociated acid in the aqueous phase. Partition coefficients of acetic, lactic, and sorbic acids between sunflower oil and water have been reported as 0.02, 0.033, and 2.15, respectively,³² demonstrating the potential for, particularly, the undissociated form of sorbic acid to decrease in the oil phase of biphasic foods.

As a complication, the pH of foods preserved using organic acids is typically in a region where weak organic acids are present in both the undissociated and the dissociated forms. Calculation of the residual concentration of the undissociated form following partition is thus difficult because the concentration is subject to the effects of partition, and to the dissociation equilibrium based on the new pH of the system and the new residual concentration of undissociated acid.

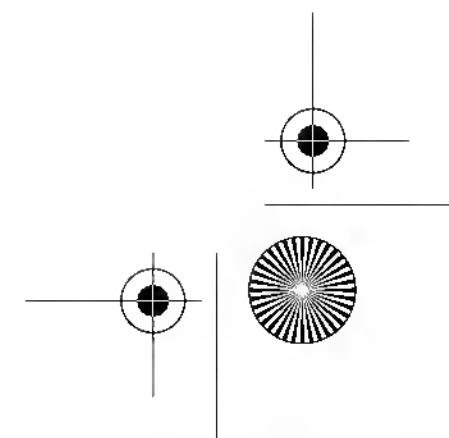
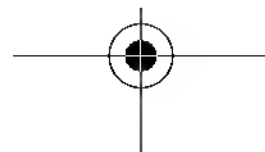
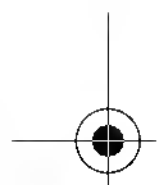
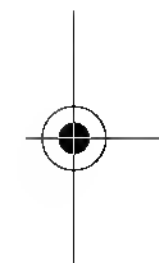
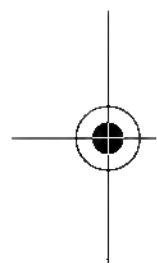
A modified form of the Henderson–Hasselbalch equation has been developed,¹⁹⁸ which takes these effects into account and gives the proportion of the total weak acid in a two-phase system that is present in its undissociated form in the aqueous phase, given the pH, the volume fraction of oil, and the partition coefficient for the undissociated weak acid. It was cast as:

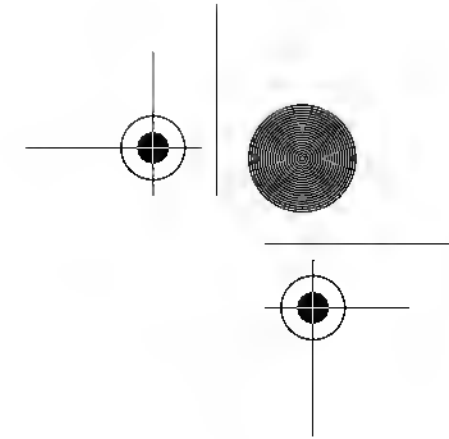
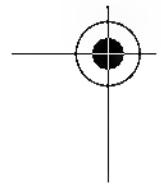
$$\frac{[\text{HA}]_{\text{aq}}}{[\text{HA}]_{\text{T}}} = \frac{1}{1 + K_p \left(\frac{\phi}{1 - \phi} \right) + 10^{(\text{pH} - \text{p}K_a)}} \quad (5.5)$$

where K_p is the partition coefficient and ϕ is the fraction volume of the oil phase. Predictions have been validated in aqueous and biphasic foods.¹⁹⁸

5.2.4 WATER ACTIVITY

Water activity (a_w) is a measure of the concentration of available water in a food and can be defined as the tendency of water to escape from a solution relative to its

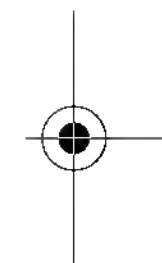
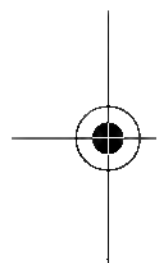




ability to escape from pure water at a specific temperature. Water activity is equal to the equilibrium relative humidity divided by 100. Pure water has an a_w of 1.000, and an environment where water is absent has an a_w of 0.000.^{49,182} Most microorganisms require a high a_w for growth, and a_w is included in many predictive microbiology models. The a_w of foods can be adjusted by the addition of solutes (humectants), such as sodium chloride, sucrose, or glycerol. In some cases, the solute itself may have toxic effects, and the inhibition of growth of microorganisms when sodium chloride is used to adjust a_w can be greater than when glycerol is used, due to the toxicity of high concentrations of sodium chloride.^{12,75,182} Care must be taken, therefore, to use only those predictive models that use the same humectant as the food of interest. Prediction of the initial a_w of the food can be achieved from first principles using a variety of equations, such as Raoult's law,^{49,93} which was derived by Christian⁴⁹ as:

$$\text{Log}_e a_w = \frac{-vm\phi}{55.51} \quad (5.6)$$

where m is the molal concentration of the solute, v is the number of ions generated by each molecule of the solute, and ϕ is the molal osmotic coefficient. Commercial software to predict water activity from a list of food ingredients in a recipe is available (e.g., ERH CALCTM).

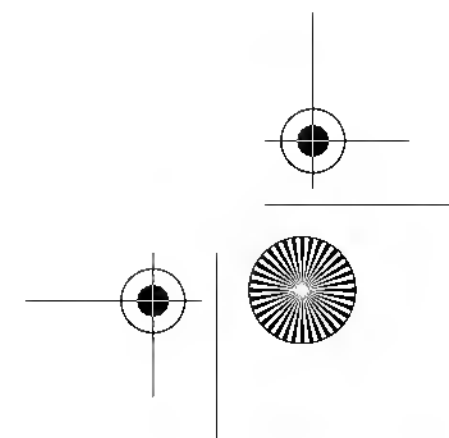
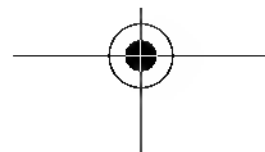
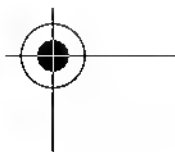
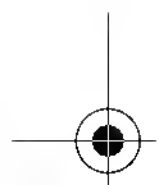


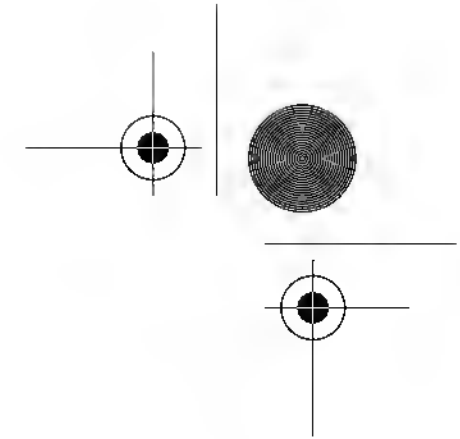
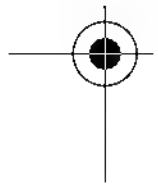
5.3 HURDLE CONCEPT

Hurdle technology involves the use of combinations of physical or physicochemical preservation techniques at subinhibitory levels to control the growth of food-borne microorganisms.⁹⁸ This has the effect of conferring microbial safety and stability while maintaining acceptable nutritional and sensorial attributes,¹⁶⁰ an approach that is important for minimally processed extended shelf life foods.¹⁰⁸ With the development of new food products that depend on multiple barriers to ensure safety, it becomes necessary to develop the means to apply predictive microbiology to hurdle technology.^{43,97} Careful definition of the conditions defining the boundaries of growth or survival will allow industry to design foods with the appropriate level of safety;^{149,160} however, there have been few attempts to provide a quantitative assessment of hurdles.¹⁶⁰

Examples of interactions include CO₂, pH, and NaCl on *L. monocytogenes*;⁷¹ temperature, pH, citric acid, and NaCl in reduced calorie mayonnaise on *Salmonella* spp.;¹²¹ pH, acid, and salt on *Staphylococcus aureus*;⁶⁴ salt, pH, and nitrite on *Escherichia coli* O157:H7 in pepperoni;¹⁵¹ temperature and pH on *E. coli* O157:H7 in Lebanon bologna;⁶⁶ and nisin and leucosin on *L. monocytogenes*.¹³⁸

While it is clear that combinations of hurdles can influence food-borne microorganisms, it is not clear to what extent these factors interact. When the square root model is used to describe the effect of several hurdles such as temperature, pH, and a_w , these factors are usually considered to act independently, with no interactions.¹¹⁹ Ratkowsky and Ross¹⁴⁹ described a combined probability/kinetic model for *Shigella*



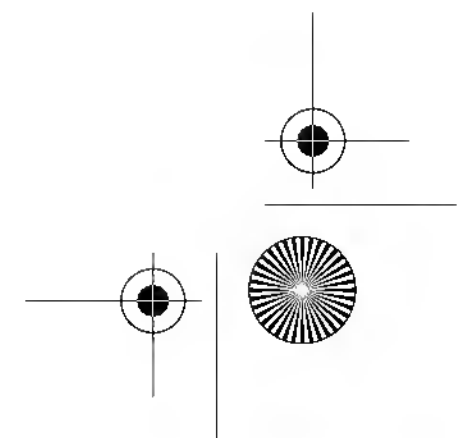
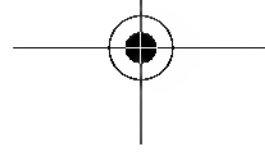
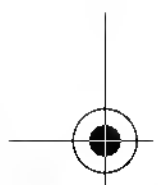


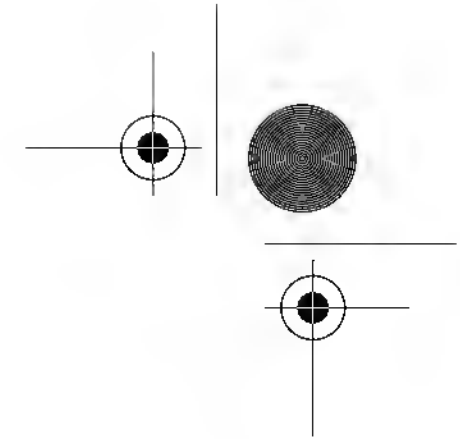
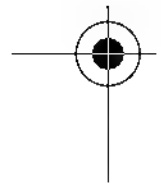
flexneri in which temperature, pH, a_w , and nitrite were shown to act individually. It would be expected, however, that interactions must occur between certain hurdles. For example, interactions between organic acids and pH would be expected (due to the influence of pH on the extent of dissociation as described in Section 5.2) and have been observed.^{38,121,147} Effects on heat resistance of *E. coli* due to the interactions between combinations of temperature, pH, NaCl, and sodium pyrophosphate have been modeled.^{86,87}

Polynomial models can be used to describe interactions between a wide variety of hurdles. This is because the regression methods used facilitate the search for quadratic or interactive effects. Combination effects have also been modeled using Belehradek and Arrhenius models.^{90,91} The growth of *L. monocytogenes* at 9°C as influenced by sodium nitrite, pH, sodium chloride, sodium lactate, and sodium acetate has been modeled,¹³⁰ and predictions compared with the growth of organisms in real sausage and predictions from Food MicroModel. Food MicroModel is a software package developed in the U.K. that contains secondary models of the effects of environmental factors (mainly pH, concentration of NaCl, and temperature) on the survival, growth, and thermal death of major food-borne pathogenic bacteria in broth. Predictions were on average within 20% of the Food MicroModel predictions based on 10 experiments although predictions of growth in sausage were, on average, 16% below the observed values based on inoculation of four sausages. This is perhaps related to the effects of structure as described in Section 5.1. The effect of previous growth temperature, previous cell concentration, and previous pH on the lag time and specific growth rate of *Salmonella typhimurium* has been investigated using response surface models.^{135–137} In all cases the previous growth history did not influence the predictions of the model.

Some authors contend that predictive models of the combined effects of temperature and water activity and the combined effects of temperature and pH suggest that the effect of the combinations on growth rate is independent.¹²⁰ However, these authors go on to state that the factors are interactive at the no-growth interface (i.e., the point where growth ceases). Such interface models quantify the probability of growth and define conditions at which the growth rate is zero or the lag time is infinite. Such new growth interface or habitat domain models have been published.^{116,181} Square root models and response surface models were developed to look at the effects of interactions between dissolved carbon dioxide and water activity on the growth and lag time of *Lactobacillus sarcae*.⁵⁸ The response surface models showed the best correlation although at low water activities, predictions were illogical. Both models, however, proved to be useful in the prediction of the shelf life of meat products, and were validated by comparison with an existing model.¹⁹⁶ Similarly, a quadratic response surface model was built to predict the combined effects of temperature and modified gaseous atmosphere on the growth of *Yersinia enterocolitica*.¹⁴³

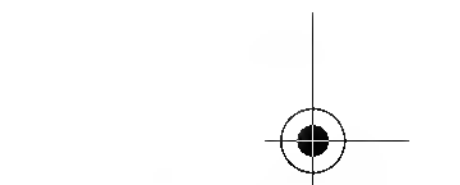
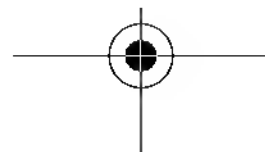
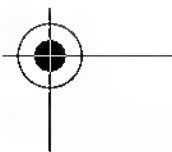
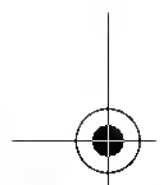
Predictive models have been used to predict the response of *Listeria monocytogenes* exposed to acid, alkaline, or osmotic shock at the time of inoculation on the subsequent effects of temperature, concentration of NaCl, and pH.⁴⁷ The authors found that predictive models were unreliable, highlighting potential problems of variable conditions, but failing to consider the implications of adaptation of the

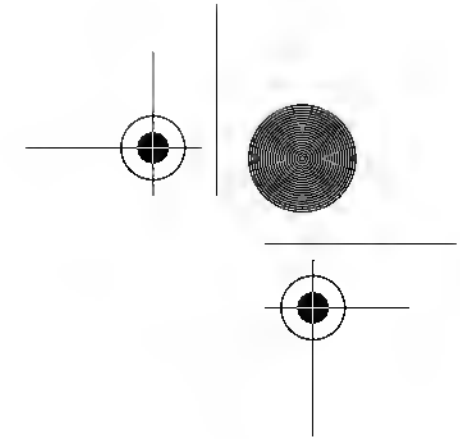
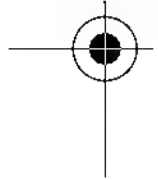




organisms to osmotic or pH effects. An important development is the use of the gamma concept, which assumes that the effects of controlling variables can be multiplied and that the cardinal parameters of temperature, pH, and water activity are not a function of other variables.¹⁹⁶ Accordingly, these authors developed a model based on the prediction of growth rate as a function of temperature and water activity and another where growth rate was predicted as a function of temperature and pH. The two models were multiplied to produce one overall model, which was validated against new experiments. Additive interaction between inhibitors has been observed.²⁴ These authors used a response surface methodology to model the response of *L. monocytogenes* to a bacteriocin (curvaticine) and sodium chloride: the model showed that the combination of the two inhibitors was greater than the effect of each individually. Interactions between inhibitory compounds were also investigated⁸ by using a series of secondary models⁷ describing independently the effects of environmental factors.⁸ The authors of the latter work then went on to show that, by taking into account interactions between environmental factors, the model decreased the frequency of fail-safe growth predictions from 13.5 to 12.1%, while the frequency of fail-dangerous no-growth predictions decreased from 16.1 to 7.1%. These findings suggest that interactions are occurring within the system, and that the models were taking them into account.⁸ However, even with multiplicative models the predictions are less accurate to describe lag time and growth rate near the limits of growth of microorganisms,⁷ and lag time models were particularly vulnerable to error.

Inactivation modeling is less common in response to a combination of hurdles. Death kinetics as a function of pH, storage temperature, and concentration of essential oil have been described using a quadratic function, and used to predict successfully the death of *Salmonella* in home-made salads.⁸⁹ A regression model describing the heat inactivation of *L. monocytogenes* was based on the Gompertz Equation.⁴⁸ The equation enabled separate characterization of the parameters of the shoulder, the maximum slope, and the tail. Interactive effects were then derived from the regression model. This showed that the shoulder region of the survival curve was affected by pH, and the maximum slope by temperature, fat content, and interaction of temperature and milk fat. Model validation was successful for temperatures only above 62°C, however. The combined effects of pH and ethanol on the heat inactivation of *B. cereus*, *S. typhimurium*, and *Lactobacillus delbrueckii* were modeled using a series of second-order polynomial equations to describe variations in *D* values resulting from changes in pH or added ethanol.⁴⁵ The heat inactivation of *B. cereus* spores was modeled using a new concept of *z*-value modeling using a *z*(pH) value,⁹⁶ where *z*(pH) was defined as the difference in pH from a reference pH value required to effect a 10-fold reduction in the *D* value. A linear relationship between the calculated *z*(pH) value and the lowest of the *pK* values of organic acids used to effect heat resistance was found. The heat resistance of *Listeria monocytogenes* in logarithmic phase cells that had been heat shocked at 42°C for 1 h and subcultures of cells that were resistant to prolonged heating has been modeled.⁹ A better fit for the survivor curves was found using sigmoidal equations compared with the classical log-linear models. Comparisons between models showed that an increase of thermal tolerance was induced by sublethal heat shock or by the selection of the heat-resistant





population. Both isothermal and nonisothermal heat inactivation effects on the germination and heat resistance of *B. cereus* spores have been modeled.⁷⁰ An inactivation model was developed for *Salmonella enteritidis*.⁹⁶ It modeled the response of the organism to a range of concentrations of oregano essential oil and temperatures at two pH values. Quadratic functions were then used to predict the growth of this organism in home-made salads. The inactivation kinetics of *E. coli* O157:H7 were modeled using the Baranyi model (based on a set of nonautonomous differential equations)¹³ as a function of time to estimate the kinetic parameters.¹⁶⁴ Quadratic models were then developed with natural logarithms taken of the shoulder and death rate as a function of temperature, pH, and concentration of oregano essential oil. The predicted values from the model were validated using viable count measurements made within real salads.

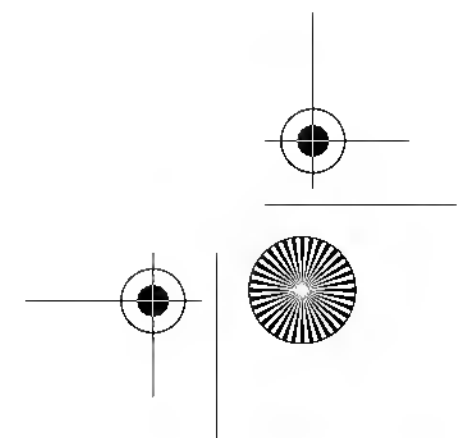
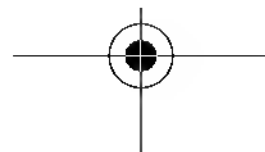
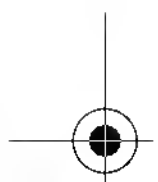
Modeling spore responses (other than inactivation) is unusual. The germination kinetics of spores of proteolytic *Clostridium botulinum* 56A as a function of temperature, pH, and concentration of sodium chloride have been modeled.⁴⁶ The germination kinetics were collected and expressed as the accumulated fraction of germinated spores with time and each environmental condition, and this accumulated fraction was then described by an exponential distribution. Quadratic polynomial models were developed by regression analysis of the exponential parameter and the extent of germination as a function of the variables under study. Validation experiments confirmed that the predictions were acceptable, and in most cases were fail-safe.

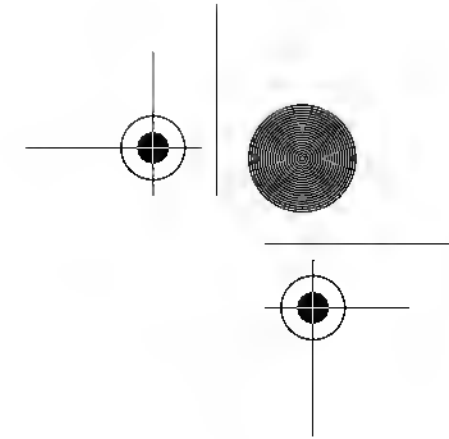
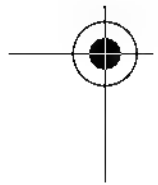
5.4 COMPETITION WITH OTHER MICROORGANISMS

Existing published models include a wide range of environmental, physical, or chemical factors; however, the competitive influence of microorganisms has not yet been incorporated into them. Competition may not be an issue in many foods, since interactions would not be expected until cell numbers had reached a potential hazard or caused spoilage.¹⁶⁰ On the other hand, growth of *L. monocytogenes* in dairy products is influenced by the natural microflora, and interactions may be difficult to model.³³ Therefore, it has been suggested that competition must be considered in the development of predictive models.¹⁶³

Competition between microorganisms in a solid matrix such as food depends to a large extent on proximity of colonies to each other.²⁰¹ Cells growing on surfaces generate gradients of redox potential, pH, oxygen concentration, and nutrients, which can influence the growth of neighboring colonies. This phenomenon can be observed in foods, for example, where “nests” of lactic acid bacteria in fermented sausage influence the survival of food-borne pathogens,²⁰¹ and also in dairy products where interactions between natural microflora and *L. monocytogenes* are influenced by the nature of the food matrix.³³

A related concept is the idea of “maximum carrying capacity” of a food product,¹⁶⁰ in which inhibition of pathogens by other microorganisms takes place when the competing flora have reached numbers at which the environment can support no further growth. This was observed with cocultures of *L. monocytogenes* and *Carnobacterium piscicola*.³⁵ In this study, the maximum population density of *L. monocytogenes* was reduced by the competing lactic acid bacteria, and this was

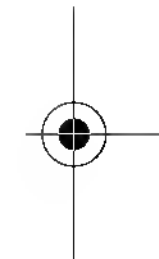
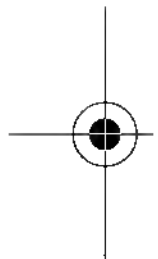




attributed to nutrient depletion. It is by no means clear to what extent competition is related to depletion of nutrients. The thermal tolerance of *S. typhimurium* was enhanced by the presence of competing microflora, and it was suggested that the presence of competitors may have influenced the pathogen to induce stationary-phase gene expression.⁶²

The interaction of spoilage microorganisms has recently been quantitated by Pin and Baranyi.¹⁴² Polynomial models were developed for a number of microorganisms, and the growth of groups of strains was compared individually and in the total mixture. This approach allowed the identification of the dominant group on the basis of its growth rate and lag time. These authors also showed that reduced growth rate could be attributed to microbial interactions. Competition from naturally occurring microflora has been documented.⁹⁴ Here, predictions of the growth of *Pseudomonas* and *Listeria* in meat were made. Predictive models worked well in predicting the growth of both organisms in decontaminated meat and in decontaminated meat inoculated with each organism, together or individually. However, the presence of naturally occurring microflora in non-decontaminated meat prevented the initiation of growth of *Listeria* and the predictive models failed.

A related aspect of interaction is that of the potential for quorum sensing between microorganisms.¹⁰¹ At low inoculation concentrations, modifications to modeling approaches were necessary to take into account inoculum size variation. Modeling the effects of inoculum size stochastics, however, confirmed that the growth rate was independent of inoculum concentration but that variability occurred as the inoculum concentration decreased.^{209,210}

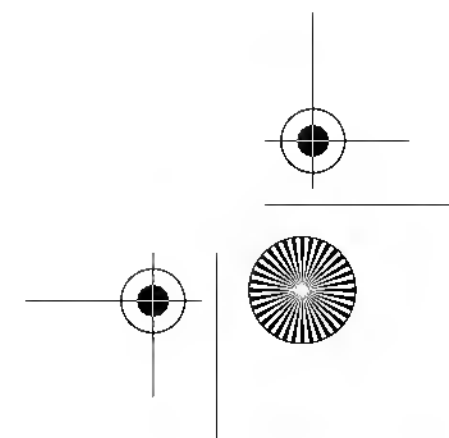
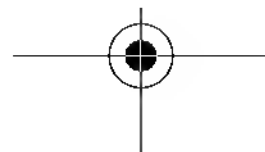
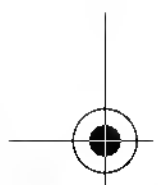


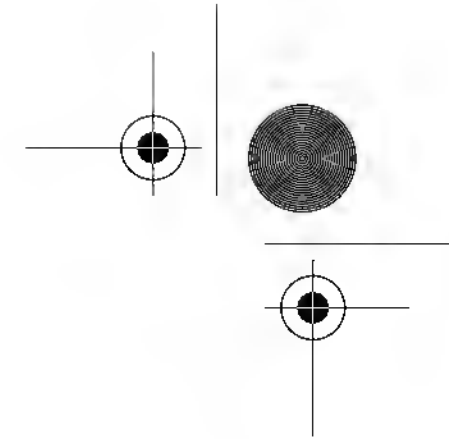
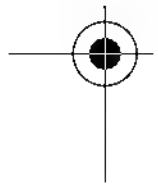
5.4.1 INTERACTIONS BASED ON THE END-PRODUCTS OF METABOLISM OF ONE SPECIES

This is a complex modeling task, but stoichiometric modeling can be used to relate the end-products of metabolism to the inhibition of the same or an accompanying organism. It assumes a “reaction scheme,” and seeks to choose the simplest representation of a system that embodies the behavior of interest.

Thus, a stoichiometric model can predict the local changes in weak acid concentration resulting from microbial growth. This must then be used to predict changes in local pH. This can be done by an empirical characterization, merely by using a titration of the growth environment with the acid of interest, and fitting a curve to these data. Alternatively a quasi-mechanistically based approach may be taken,¹³² or use made of a Buffering theory¹⁹⁸ described in Section 5.2. An advantage of the latter is that the model may be easily applied to systems of differing buffering capacity, and can combine the effects of mixtures of weak acids. Diffusion is an integral part of such modeling, and a standard model of Fickian diffusion using published diffusion coefficients in aqueous solution is usually appropriate.

For growth in liquid systems, a cardinal growth model has been combined with cardinal pH data.⁹⁹ Cardinal models use the cardinal values (minimum, optimum, and maximum values) of the environmental factors that constrain growth. Instantaneous growth rates from this model were used in a modified Baranyi growth model,¹³ together with stoichiometric parameters determined from bioreactor experiments.¹⁹⁷



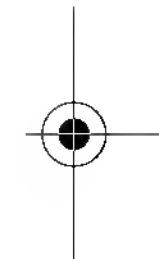
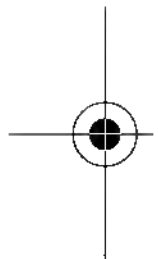


The change in pH from production of lactic acid was determined by use of a Buffering theory.¹⁹⁸ Very close agreement was found between the model and the data.

5.4.2 MIXED CULTURE

Application of stoichiometric approaches to mixed cultures also works well. Wilson et al.¹⁹⁷ showed the growth of a mixed culture of *Lactococcus lactis* and *Listeria innocua* in a bioreactor at pH 4.5. Predictions used cardinal model parameters,⁹⁹ and stoichiometric parameters from bioreactor experiments.¹⁹⁷ A Buffering theory¹⁹⁸ was used to predict changes in pH. Such an approach provided good prediction of both the rate and extent of growth of the two organisms. Of interest in these approaches is that a stationary phase was not incorporated into the primary growth model, but emerged from the prediction in response to the accumulation of metabolites.

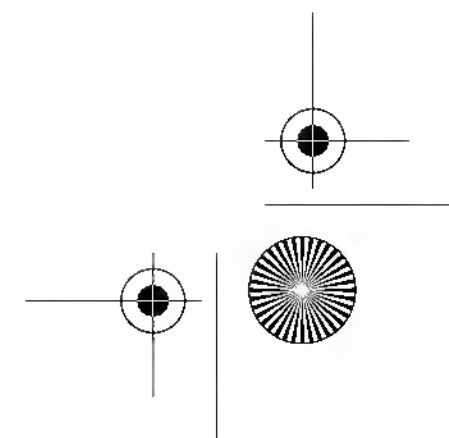
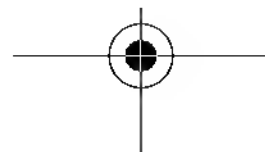
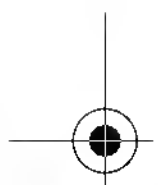
Interactions resulting from the production of antimicrobial bacteriocins by lactic acid bacteria in conjunction with the inhibition resulting from production of lactic acid have been modeled.⁴⁴ These authors used a modification to logistical equations that described the combined (although not additive) effects of two or more inhibitory compounds. They then applied their findings to the inhibition of *Leuconostoc mesenteroides*. The inhibition of growth of *Enterobacter cloacae* by *Lactobacillus curvatus* resulted from the production of lactic acid by the latter, and the concomitant decrease in pH,¹⁰⁵ which was also inhibitory to *L. curvatus*. This interaction has been modeled using a set of first-order differential equations describing growth, consumption, and production rates for both microorganisms.¹⁰⁷ Parameters were obtained from pure culture studies and from the literature, and the equations were solved using a combination of analytical and numerical methods. Predictions of growth of mixed cultures used parameters from pure culture experiments, which were close to the experimental data. The models also showed that interactions occurred when the antagonistic bacterium, in this case *L. curvatus*, reached 10^8 cfu/ml.

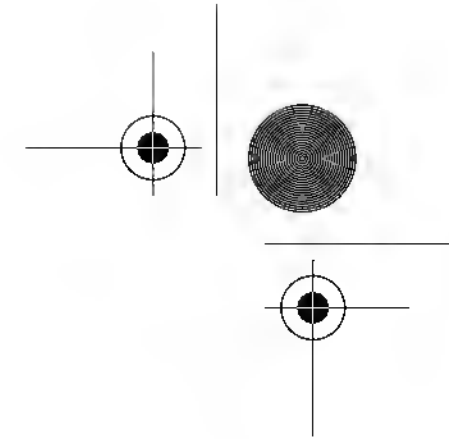
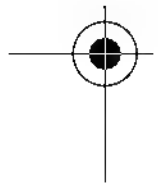


5.5 ADAPTATION AND INJURY

5.5.1 EFFECTS OF ENVIRONMENT ON ADAPTATION

Predictive microbiology should deal with bacterial stress within populations.⁶ An example is the extension of the lag time of *Listeria monocytogenes* under suboptimal conditions when the inoculum was stressed.⁶ More important, considerable interest has arisen recently in the problems of adaptive responses of bacteria and in the cross-resistance that this can confer. For example, adaptation of bacteria to methods of preservation can result in survival or growth that is better than predicted if the adaptive response is ignored. Accordingly, adaptation of bacteria can lead to unsafe or spoiled food.³⁴ The implications of adaptation can be demonstrated by reference to the acid tolerance response (ATR). The ATR in *L. monocytogenes* has been attributed to the *de novo* synthesis of proteins (sometimes referred to as acid shock proteins) when exposed to a decrease in extracellular pH.¹³⁴ Such biochemical changes confer acid resistance on the organisms, but O'Driscoll et al. also noted





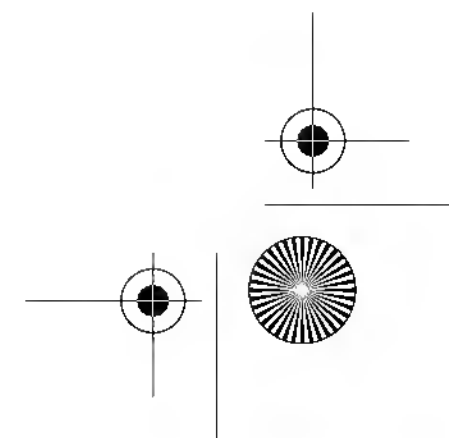
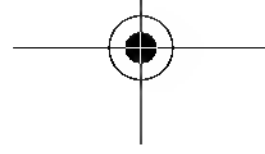
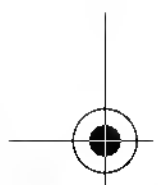
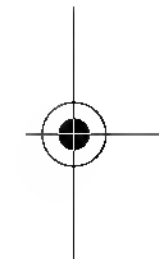
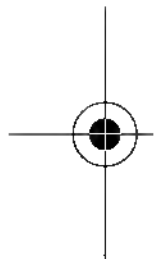
that *L. monocytogenes* that had been induced to show the ATR also had an increased resistance to thermal, osmotic, and cold stresses.¹³⁴ ATR has been defined as the resistance of cells to low pH when they have been grown at moderately low pH or when exposed to a low pH for some time,⁵⁹ and is typically demonstrated in broth culture, where a pH of 4.8 to 5.0 is reported to give an optimum ATR.⁵⁶ Many foods fall into this region of pH, and, more important, many microorganisms can experience this pH transiently during food production or sanitation protocols. Adapted populations could then result.

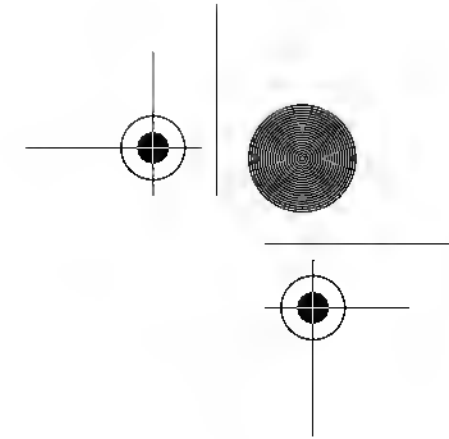
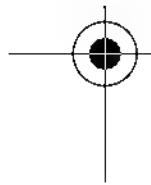
Additionally, it is clear from the above sections that one of the key effects of food structure is the immobilization of microorganisms and their resultant growth as colonies. This results in local changes in the concentration of substrates²⁰¹ and, particularly, a local accumulation of acidic metabolic end-products leading to a decline in pH within and around the colony^{104,192} with a pH gradient extending into the surrounding medium.^{192,201} In the case of *S. typhimurium*, the pH gradient extended from the original pH 7.0 in the surrounding medium to pH 4.3 inside the colony.¹⁹² Such a local decline in pH within the colony is greater than the change required to stimulate an ATR in *Salmonella* and other Gram-negative enteric bacteria⁹⁵ and in *L. monocytogenes*.⁹² It is conceivable, therefore, that cells of food-borne pathogenic bacteria immobilized as colonies embedded in a food matrix may undergo a self-induced ATR stimulated by a localized pH that has declined by virtue of the colony's own metabolic processes. It is known that acid shock proteins are synthesized and exported from cells experiencing adaptation in broths. Should this also be the case in colonies, it would result in cells within the colony becoming acid tolerant.

Despite the importance of adaptation in food microbiology, attempts to model it are rare. Authors have acknowledged that organisms behaved differently when exposed to changes in pH or sodium chloride concentration, and that exposure to these agents during exponential phase had a more dramatic effect than during the lag phase when adaptation was possibly induced.⁴⁷ However, no attempt to incorporate adaptive responses into models was made. A cross-resistance between high hydrostatic pressure and mild heat, acidity, oxidants, and osmotic stresses was demonstrated for *E. coli* O157.²⁰ Differences were most dramatic in stationary-phase cells; the only exception being acid resistance where differences were also apparent in the exponential phase, although, again, no attempt to incorporate these into a model was made. In one attempt to model adaptation, a model to describe the influence of temperature and the duration of preincubation on the lag time of *L. monocytogenes* was developed.¹⁰

5.5.2 EFFECTS OF SUBLETHAL INJURY

Subjection of bacteria to inimical processes can result in the cumulative injury of the bacteria, resulting in death. Sublethal injury is the reversible damage inflicted on bacteria that is insufficient to cause a loss of viability, and from which the bacteria can recover.^{5,80,150} It is an important phenomenon to recognize when collecting data for modeling, because bacteria can often fail to form colonies on conventional selective microbiological culture medium used for their enumeration.^{2,127} They can

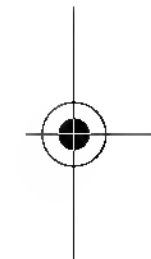
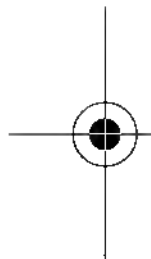




also fail to respond positively to viability stains.²⁶ However, the cells can remain viable and the injury can be repaired in foods, where the bacteria can then increase in numbers.^{82,206} The severity of treatment that results in sublethal injury differs between species, although serotypes of *Salmonella* have been found to respond similarly to one another.¹²⁸

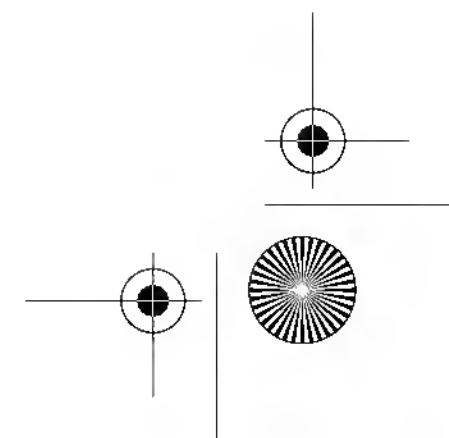
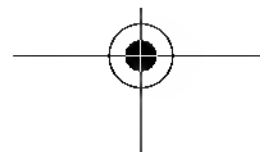
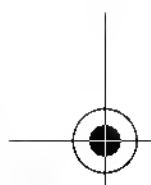
5.5.2.1 Enumeration of Sublethally Injured Bacteria

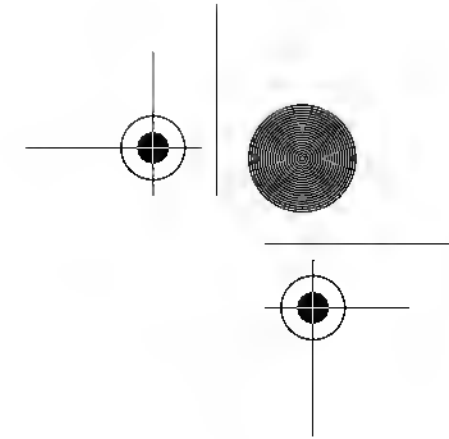
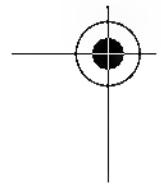
A range of methods have been used to determine the extent of injury of microorganisms. These include differential plate counts on selective and nonselective agars^{15,150} or on minimal and more complex media,¹⁰² extension of the lag phase,^{4,102} and changes in bioluminescence.⁶⁷ Such methods can be used to optimize both the recovery medium and the time and temperature of incubation. For example, it has been shown that cells of *L. monocytogenes* that were subjected to sublethal injury by heat exhibited a broad optimum temperature for recovery, with an optimum between 20 and 25°C, but that incubation at 2 or 5°C failed to allow repair.¹⁰³ The time taken for repair of injury to complete can be determined by measuring the time before equivalent counts are found on a selective medium (which will not support the growth of sublethally injured bacteria) and a nonselective culture medium (which will allow the growth of sublethally injured bacteria).¹⁰³ Some modeling of resuscitation has been published.¹¹⁷ Predictions of response might be possible: for example, a relationship was found between the concentration of sodium chloride in the heating menstrum and its concentration in the growth medium used for the resuscitation and subsequent enumeration of *S. typhimurium*.¹⁰⁶



5.6 VALIDATION IN FOODS

One of the most important aspects of model development is ensuring that predictions made by the model are applicable to real situations. This is the validation process. It should involve comparisons of the predictions of the model with observed measurements, which should be different data to those used to construct the original model. Although some predictive models have been constructed in real foods (see later in this chapter), the vast majority of models have been constructed from experiments performed in laboratory culture media (typically broth). In all cases the validation process should, ideally, include comparisons with the behavior of microorganisms in real foods or during real food processes. However, due often to cost but also other factors, validation can be done in model systems, or using previously published data. A validated model should be consistently “fail-safe,” that is, predictions should fail on the side of safety (i.e., predicted growth rate and lag time should be faster and shorter, respectively, than experimental values). Predictive models can be crucial aspects of HACCP protocols. Imaginary scenarios depicting the way in which predictive models can be incorporated into HACCP concepts have been published,¹²² as has a useful review of the application of predictive food microbiology in the meat industry.¹¹³ Similarly, predictive microbiology is an important element of Quantitative Microbial Risk Assessment (QMRA). Models are useful decision support tools, but it should be remembered that models are, at best, only a simplified





representation of reality. The application of model predictions should be tempered with previous experience and with knowledge of other microbial ecology principles that may be experienced in the food by the organism.¹⁵⁸ Sources of data and models relevant to the growth of *L. monocytogenes* in seafood and that could be part of a QMRA have been published.¹⁵⁸

5.6.1 BIAS AND ACCURACY

Some criticism of the term “validation” revolves around the difficulty in quantifying just how well models perform their predictive role. Error occurs implicitly in the use of data for modeling and the use of those models for the prediction of growth of microorganisms. There are a number of potential sources of error: the homogeneity of foods; the completeness of the environmental factors used to collect the data; conversion of empirical results to a mathematical function; and fitting the models to the data.¹⁵⁹ For example, the overall errors in the application of growth models to the growth of *Pseudomonas* species in food and in laboratory media have been quantified.¹⁴⁴ The authors made the point that the error was small in the case of culture medium but great in the case of food, and went on to quantify the influence of food structure and composition on the overall error. Sutherland et al.¹⁷² found that much of the published work on *E. coli* O157:H7 was done under conditions outside of the experimental values used to develop their growth model. These workers also reported that validation with data from cheeses and meats was difficult because the original authors often did not report experimental conditions such as NaCl content or pH. In these cases, poor predictions were often made. Similar observations were made when a growth model for *B. cereus* was being validated.¹⁷⁰

It is clear in the above cases that some quantification of the deviation of the predictions from the observed values would be useful. Many measures of such quantification of error in the validation process have been made.¹⁵⁷ Additionally, however, Ross¹⁵⁷ has proposed using simple indices of the performance of models as a step towards an objective definition of the term “validated model.” These indices give an indication of the confidence with which those models can be used (accuracy factor), and whether the model displays bias towards fail-dangerous predictions (bias factor). The accuracy factor is defined as:

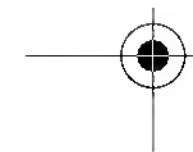
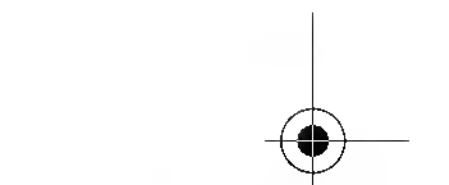
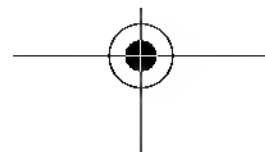
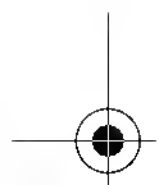
$$\text{Accuracy factor} = 10^{(\sum |\log(GT_{\text{predicted}}/GT_{\text{observed}})|/n)} \quad (5.7)$$

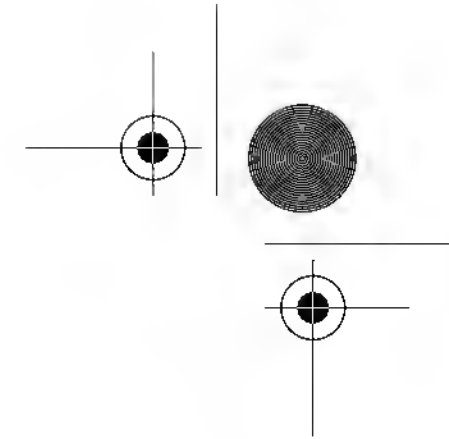
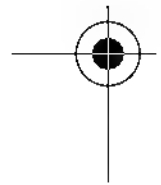
where $GT_{\text{predicted}}$ is the predicted generation time and GT_{observed} is the observed generation time, and n is the number of observations. The less accurate the predictions the larger the accuracy factor.

The bias factor is defined as:

$$\text{Bias factor} = 10^{(\sum \log(GT_{\text{predicted}}/GT_{\text{observed}})/n)} \quad (5.8)$$

If no disagreement between predicted and observed values occurs then the bias factor is equal to 1. However, a value of the bias factor greater than 1 indicates a fail-





dangerous model because it will predict generation times longer than actually observed. It should be noted, however, that when rate values are used to compute the bias factor, a fail-dangerous model will have a bias factor of less than 1.

As mathematical techniques advance, so does the process of comparing models. The use of artificial neural networks has been identified as a useful alternative technique for modeling microbial growth. Neural networks also lend themselves to quantifying comparisons between models and suitable indices have been suggested.⁸⁵

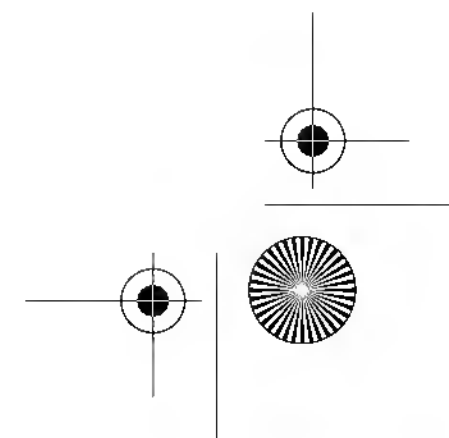
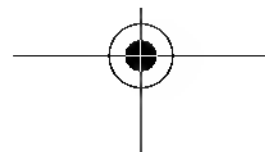
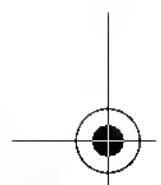
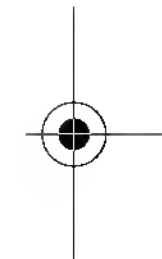
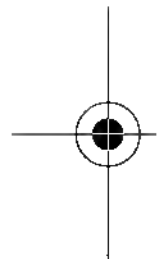
5.6.2 VALIDATION USING LITERATURE VALUES

The most common method of validation is the use of literature data. This is based on the assumption that if the published experiments were performed under well-defined conditions that do not differ markedly from those used to develop the model, then the model predictions should be reasonably reflected in the published data. A large number of models have been validated using published information including models for *Y. enterocolitica*,^{22,100,171} *Aeromonas hydrophila*,¹¹² *Clostridium botulinum*,⁷⁶ *S. enteritidis*²³ and *E. coli* O157:H7,^{23,173} *L. monocytogenes*,^{71,111} and a number of other microorganisms.⁵⁵

There are, however, some potentially serious limitations to the use of literature data for validation of predictive models. Additional food components are frequently responsible for deviations between predicted and observed values in validation experiments. For example, Tienungoon et al.¹⁸¹ predicted the growth limits of *L. monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. The authors used two strains of *L. monocytogenes*, Scott A (a pathogenic strain) and L5 (a wild-type strain isolated from cold-smoked salmon). Experiments were carried out in broth culture at a wide range of environmental conditions. Aliquots of the inoculated media were observed for a period of 90 days to determine whether the conditions supported growth. Data from the experimental program were modeled using a probability model for growth. Figure 5.2 shows the growth boundary predicted by the model for the case of no added lactic acid, and a water activity of 0.992 (representing 0.5% NaCl in a typical culture medium) as a function of temperature and pH. This boundary is plotted alongside the data from the literature (Table 5.3). Generally, the model predicted values that were in good agreement with literature values. However, where deviation from the observed measurements occurred, this was usually explained by additional identifiable preservative factors in the system, and these are described in Table 5.3.

A similar issue arises using the growth boundary model of McKellar and Lu,¹¹⁶ which predicts the growth limits of *E. coli* O157:H7 as a function of temperature, pH, NaCl, sucrose, and acetic acid. These authors used five strains of *E. coli* O157:H7 growing in broth culture for a period of 72 h to determine whether the conditions supported growth. Data from the experimental program were modeled using a probability model for growth.

This boundary is plotted alongside the data from the literature (Table 5.4) in Figure 5.3. As above, the model predicted values that were in good agreement with literature values. Again, however, deviation from the observed measurements occurred, due to additional identifiable preservative factors, which are described in Table 5.4.



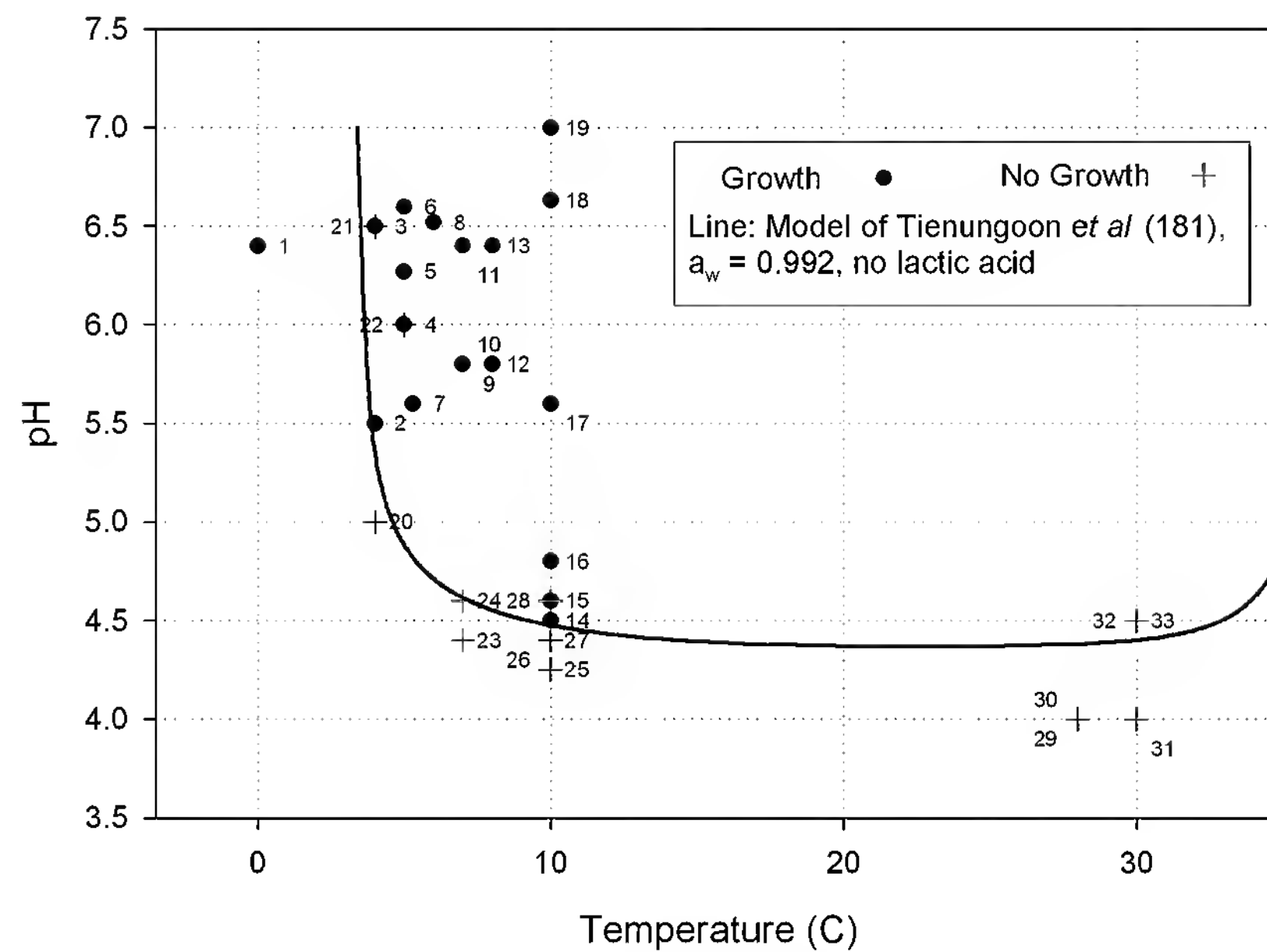
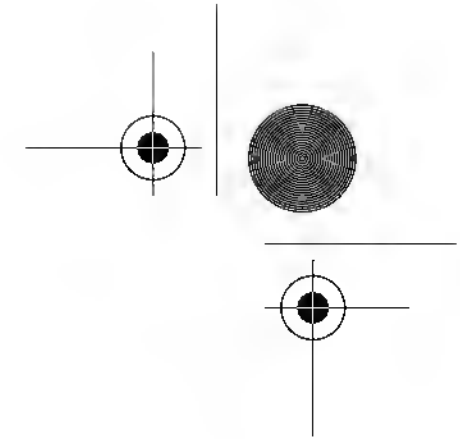
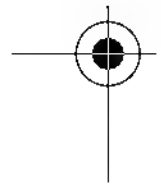


FIGURE 5.2 The growth boundary predicted by the model of Tienungoon et al.¹⁸¹ for the case of no added lactic acid, and a water activity of 0.992 (representing 0.5% NaCl in a typical culture medium) as a function of temperature and pH. This boundary is plotted alongside the data from the literature described in Table 5.3.

Other sources of error associated with the use of literature data include lack of information on preincubation conditions that might result in the development of acid tolerance; use of selective media for enumerating microorganisms; lack of estimates on variability; and presence of factors in foods that are not taken into account in models (e.g., preservatives).³⁹ It appears that the most appropriate method for validation might be to use data derived under well-controlled conditions, so that the model's performance will not be unfairly biased.¹⁵⁷ Unsafe predictions and lack of published information on error also limit the usefulness of literature data, and emphasize the need to validate against new data.⁵⁷

5.6.3 VALIDATION IN FOODS

The most common method for validating models using new data is to carry out experiments directly in the food product of concern. Thus, several models have been validated directly in food products including survival of *L. monocytogenes* in uncooked-fermented meat,¹⁹⁵ fishery products,¹⁵⁸ or pâté;⁶⁹ survival of *Campylobacter jejuni* in a variety of foods;⁵⁴ growth of *L. monocytogenes* in dairy products;¹²⁹ growth of *L. innocua* in Bologna-type sausage;⁸¹ growth of *Staphylococcus aureus* in sterile foods;¹⁹⁰ growth of *E. coli* O157:H7 on raw ground beef;¹⁸⁸ growth of *L. monocytogenes* in sterile foods;¹⁸⁹ growth of *Shigella flexneri* in sterile foods;²⁰⁷ growth of *E. coli* on raw displayed pork;⁷³ growth of *Y. enterocolitica* in seafood;¹⁴³ and growth of *Listeria* in a range of foods.¹⁷⁴

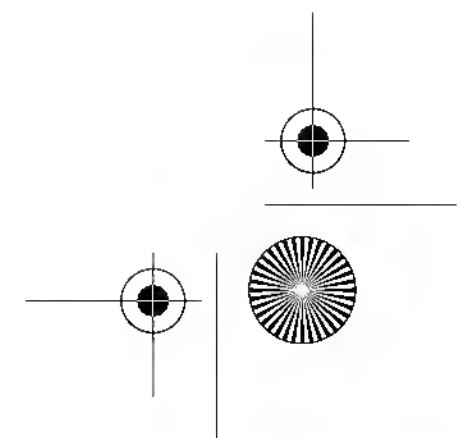
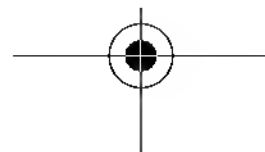
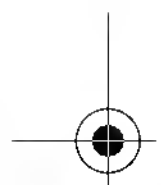


TABLE 5.3
Literature Values Used in the Validation of the Growth Boundary Model
Shown in Figure 5.2

Data Ref.	Temp. (°C)	pH	Other Hurdles ^a	Matrix ^b	Ref.	Obs. Time ^c
<i>Listeria monocytogenes</i> — Growth Data						
1	0	6.4		Chicken broth	191	
2	4	5.5	0.5% NaCl	TSBYG	71	
3	4	6.5	4% NaCl	TSBYG	71	
4	5	6	4.5% NaCl	Tryptose phosphate broth	42	
5	5	6.27	0.05% NaCl	Minced beef	111	
6	5	6.6	0.05% NaCl	UHT milk	111	
7	5.3	5.6	0.05% NaCl	Vacuum packed lean beef	111	
8	6	6.52	0.05% NaCl	Chicken legs	111	
9	7	5.8	0.05 [0.004]% acetic acid ^d	Tryptose broth	3	
10	7	5.8	0.05% citric acid	Tryptose broth	3	
11	7	6.4	0.05% NaCl	Nonfat milk	111	
12	8	5.8	0.05% NaCl	Minced beef	111	
13	8	6.4	0.05% NaCl	Skimmed milk	111	
14	10	4.5		TSBYE	181	
15	10	4.6	Poised with citric acid	TSB	167	
16	10	4.8	Poised with lactic acid	TSB	167	
17	10	5.6		Tryptic meat broth	16	
18	10	6.63	0.277% NaCl + 170 ppm nitrite	Vacuum packed ham	111	
19	10	7	$a_w = 0.96$	Tryptic meat broth	16	
<i>Listeria monocytogenes</i> — No Growth Data						
20	4	5		TSBYG	72	28 d
21	4	6.5	8% NaCl	TSBYG	71	70 d
22	5	6	4.5% NaCl + nitrite	Tryptose phosphate broth	42	NS
23	7	4.4	0.2% citric acid	Tryptose broth	3	400 h
24	7	4.6		TSBYG	72	28 d
25	10	4.25		TSBYE	181	NS
26	10	4.4		TSBYG	72	28 d
27	10	4.4	Poised with citric acid	TSB	167	28 d
28	10	4.6	Poised with lactic acid	TSB	167	28 d
29	28	4	6 [5.12]% acetic acid	BHI	40	62 d
30	28	4	9 [3.78]% lactic acid	BHI	40	62 d
31	30	4	0.029% citric acid	TSBYE	51	42 d
32	30	4.5	0.068 [0.043]% acetic acid	TSBYE	51	42 d
33	30	4.5	0.043 [0.008]% lactic acid	TSBYE	51	42 d

Note: NS = Not stated.

^a These are responsible for the deviation of the data points from the growth boundary predicted by the model.

^b The following matrices refer to commonly used microbiological growth media: TSBYG; Tryptose-phosphate broth; Tryptose broth; TSBYE; TSB; Tryptic meat broth; BHI.

^c Time for which no growth was observed.

^d Concentration of acetic and lactic acids expressed as total, with undissociated in square brackets.

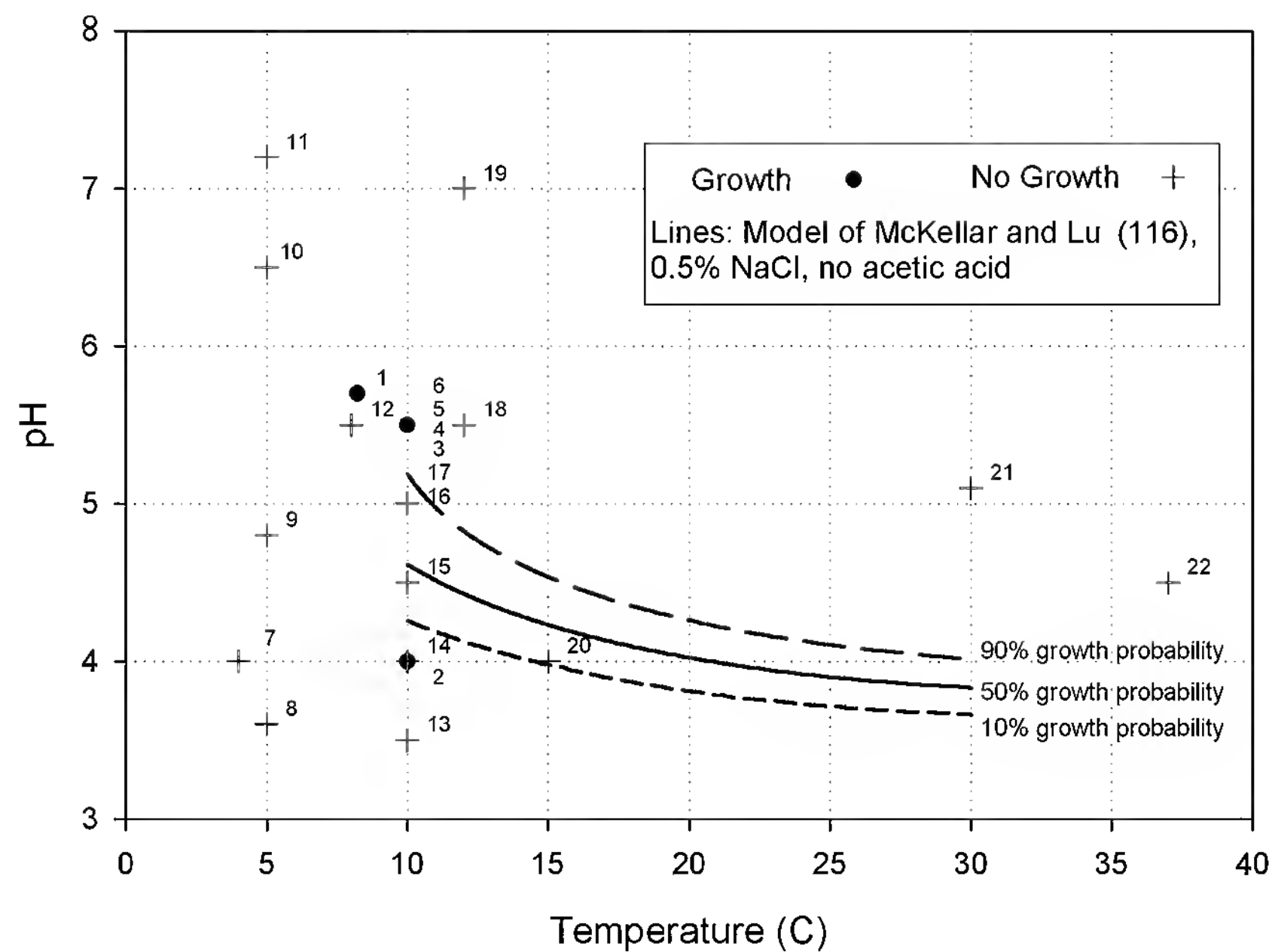
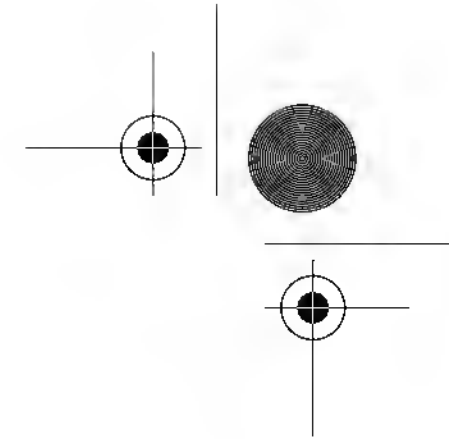
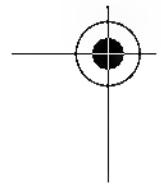
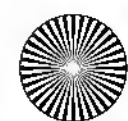
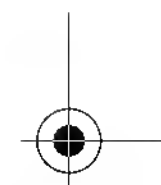
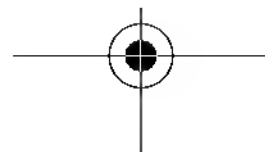
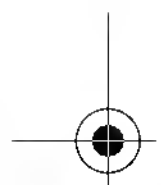


FIGURE 5.3 The growth boundary predicted by the model of McKellar and Lu¹¹⁶ that predicts the growth limits of *Escherichia coli* O157:H7 as a function of temperature, pH, NaCl, sucrose, and acetic acid. This boundary is plotted alongside the data from the literature described in Table 5.4.

Dynamic modeling has also been validated,²⁵ where predictions from FoodMicroModel have been applied to the growth of *L. monocytogenes* and *Salmonella* in a range of foods incubated under constant as well as fluctuating temperatures. The authors found that generally the accuracy of prediction under the fluctuating temperatures was similar to the isothermal conditions, although inhibition by natural microflora did decrease the expected growth of *L. monocytogenes* in milk. Significant deviation of predictions of the growth of bacteria growing as colonies when immobilized in gel occurred when predictions were made from isothermal growth in broth.^{125,126}

Validation of combined growth of the spoilage bacteria *Pseudomonas*, *Shewanella putrefaciens*, *Brochothrix thermosphacta*, and lactic acid bacteria was made in modified atmosphere packaged fish as a function of temperature and concentration of carbon dioxide.⁹¹ Combined models based on polynomial, Belehradek, and Arrhenius equations were developed and validated by comparison with experimental growth rates of these bacteria obtained on three Mediterranean fish species. Predictions of the models based on the Belehradek and Arrhenius equations were judged satisfactory overall. This approach has been modified⁹⁰ to determine a procedure for modeling the shelf life of fish. Similarly, a quadratic response surface model has been used to describe the maximum specific growth rate of *Y. enterocolitica*. The model predicted growth rates as a function of refrigeration temperature and



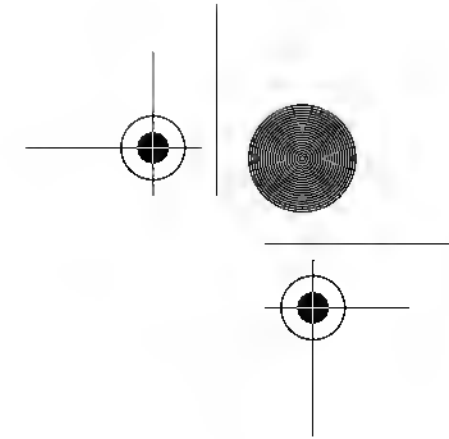
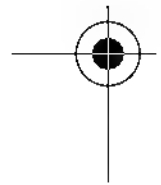


TABLE 5.4
Literature Values Used in the Validation of the Growth Boundary Model
Shown in Figure 5.3

Data Ref.	Temp. (°C)	pH	Other Hurdles ^a	Matrix ^b	Ref.	Obs. Time ^c
<i>E. coli</i> — Growth Data						
1	8.2	5.7		Ground mutton	83	
2	10	4	0.5% NaCl	TSB	116	
3	10	5.5	Poised with lactic acid	TSBYE	52	
4	10	5.5	Poised with citric acid	TSBYE	52	
5	10	5.5	0.5% NaCl	BHI	37	
6	10	5.5	5% NaCl	BHI	41	
<i>E. coli</i> — No Growth Data						
7	4	4		TSBYE	52	21 d
8	5	3.6		“Condiments”	183	7 d
9	5	4.8	$a_w = 0.99$	TSB	156	48 h
10	5	6.5	5% NaCl	BHI	41	12 d
11	5	≈7.2		Cucumber slices	1	10 d
12	8	5.5	0.5% NaCl	BHI	37	10 d
13	10	3.5	0.5% NaCl	TSB	116	72 h
14	10	4	Poised with acetic acid	TSBYE	52	21 d
15	10	4.5	5% NaCl	BHI	41	12 d
16	10	5	Poised with lactic acid	TSBYE	52	21 d
17	10	5	Poised with citric acid	TSBYE	52	21 d
18	12	5.5	30% sucrose, $a_w = 0.972$	BHI	36	24 h
19	12	≈7		Shredded carrot	1	10 d
20	15	4	0.5% NaCl	TSB	116	72 h
21	30	5.1	0.1 [0.03]% acetic acid as vinegar ^d	Nutrient agar	68	4 d
22	37	4.5	Poised with lactic acid	TSBYE	74	14 d

Note: NS = Not stated.

^a These are responsible for the deviation of the data points from the growth boundary predicted by the model.

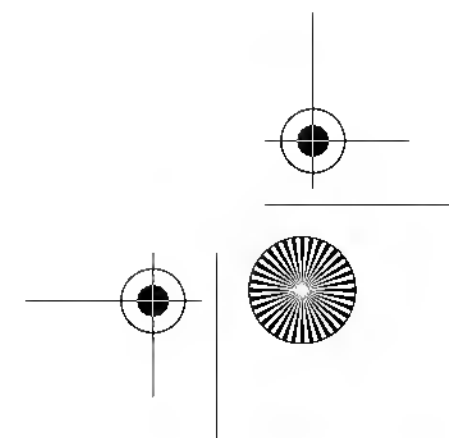
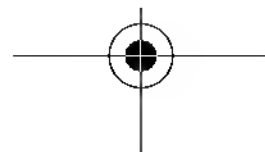
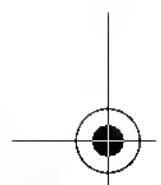
^b The following matrices refer to commonly used microbiological growth media: TSBYE; TSB; BHI; Nutrient agar.

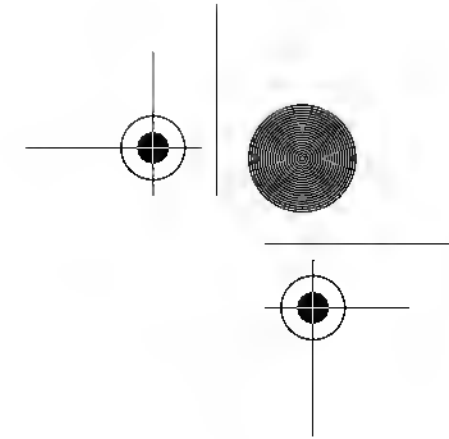
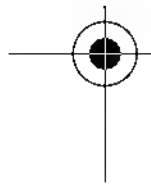
^c Time for which no growth was observed.

^d Concentration of acetic acid expressed as total, with undissociated in square brackets.

modified atmosphere and comparisons of the model predictions were made with growth rates obtained in seafood deliberately inoculated with *Y. enterocolitica*.¹⁷

Validations of the growth of *L. monocytogenes* in tryptose phosphate broth and in chicken and in beef have been made as a function of changing the pH and sodium chloride concentration.¹³³ Predictions of the growth of *L. monocytogenes* were then made using either a square root model¹⁴⁸ or a response surface polynomial model.⁴² The square root model predicted growth rates at between 0 and 25°C with a





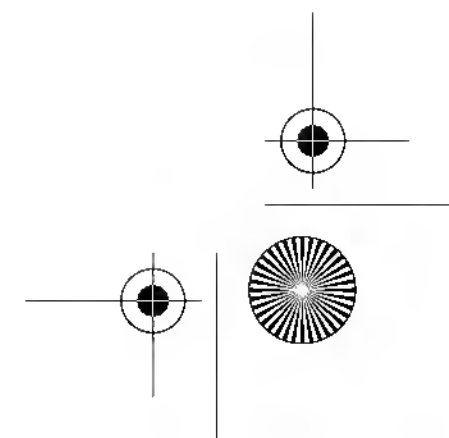
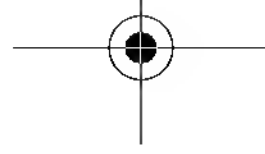
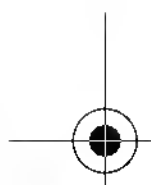
coefficient of determination of between 98.36 and 99.63%. The response surface polynomial model, however, predicted generation times at 5 to 25°C with between 0 and 17.4% difference between the observed and expected generation times in broth. Of greater significance in terms of validation in food here are the large differences observed in the generation time at pH 5.6 and 8°C (25.5 h) and the generation time predicted by the Pathogen Modeling Program (PMP) in these conditions in tryptose phosphate broth (5.3 h). The PMP is a web-based package developed in the U.S. that contains secondary models of the effects of environmental factors (mainly pH, concentration of NaCl, and temperature) on the survival, growth, and inactivation of major food-borne pathogenic bacteria in broth. A divergence from predicted values was also shown at temperatures between 0 and 3.5°C in the square root model.

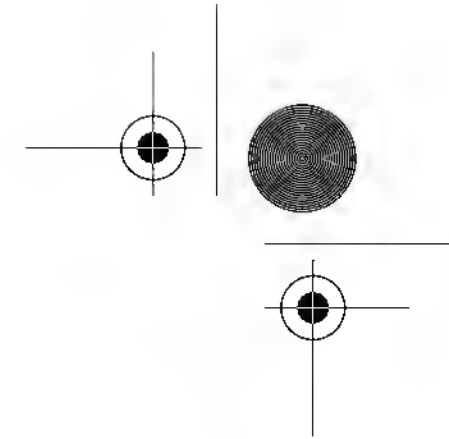
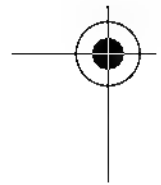
Predictions of the growth of *Bacillus cereus* from PMP were validated for its growth from spores in boiled rice.¹¹⁴ An analysis of variance showed that there was no statistically significant difference between the observed and measured growth rates in boiled rice and predictions made from PMP. Modeled predictions were fail-safe for generation time and exponential growth rate at all temperatures. Although the model was fail-safe for lag phase duration at 20 and 30°C, it was not at 15°C.

Modeling the growth of filamentous fungi is rare. The growth of three strains of heat-resistant fungi, as influenced by water activity adjusted using sucrose was modeled using the Baranyi model¹³ to fit the changing colony diameter.¹⁸⁵ Modeling the growth of filamentous fungi has also been done using a model derived from the cardinal model family. The model was successfully fitted on data sets from a range of filamentous fungi whose growth was affected by a range of humectants including sodium chloride, glucose/fructose as a mixture, and glycerol and at different pH values. Further cardinal values were extracted from the literature and the model was used to predict the evolution of the radial growth of *Penicillium roqueforti* and *Paecilomyces variotii*.¹⁶²

In spite of the effort expended to develop and validate models, it is rare to find a model developed in broth that accurately predicts behavior in food systems. Models tend to fail-safe, and provide somewhat conservative predictions.^{23,54,73,81,100,114,129,170,172} Indeed, the use of faster-growing strains has been suggested to provide a margin of safety.^{123,131,194} Although many validations of models show that there is a fail-safe tendency and hence a margin of safety in growth prediction, some manufacturers of foods find that the error is unacceptable and the margin of safety provided by such models may well be more conservative than is desirable for many food applications. There are, however, examples of situations where the model makes what are clearly unsafe predictions, and these usually involve an overestimation of the extent of lag time.^{69,188-190}

An alternative approach is to develop models directly in food products. This is not possible in many cases, due to the requirement of appropriate facilities for incorporating pathogens into the process under carefully controlled conditions. In spite of this limitation, models have been developed for growth of *L. monocytogenes* on vacuum-packed cooked meats⁶³ and liver pâté;⁶⁹ inactivation of *Salmonella typhimurium* in reduced calorie mayonnaise;¹²¹ inactivation of *Enterobacteriaceae* and clostridia¹⁸ and growth of *Lactobacillus* spp. in dry fermented sausage;¹⁹ growth of



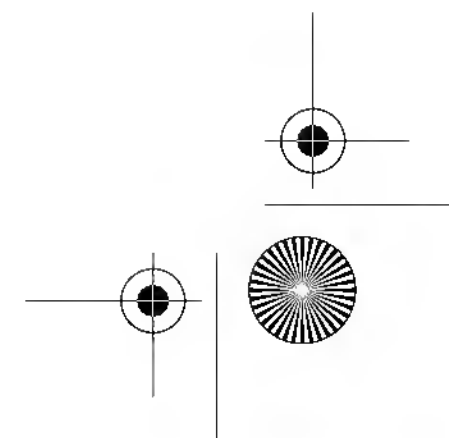
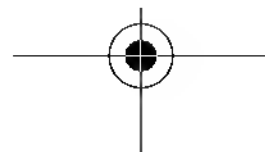
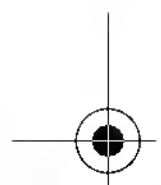
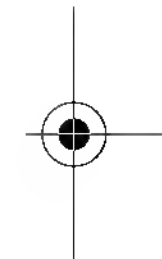
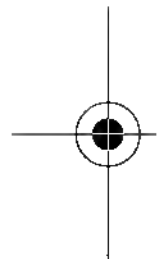


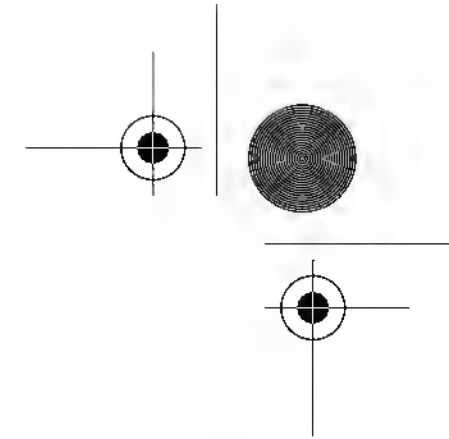
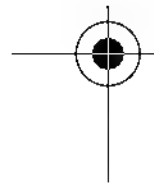
Clostridium botulinum in processed cheese;¹⁶⁹ thermal inactivation of *L. monocytogenes*¹⁴⁵ and *Enterococcus faecium*¹⁶¹ during high-speed short-time pasteurization; and the thermal inactivation of *E. faecium* during cooking of Bologna sausage.²⁰⁸ These models generally provide good estimates of the behavior of food-borne pathogens in food processes. However, it is questionable if effort should be expended developing models specific for all food processes. Improved validation techniques for models derived in broth or other model systems would appear to have more general applicability.

It has been suggested that models should only be regarded as first estimates of the behavior of pathogens, and that additional studies with products giving poor predictions should be undertaken.¹⁹⁵ Inclusion of additional data into models will often improve their predictive ability²⁰⁷; however, it is important that users of these models take great care in their use, and ensure that predictions are carefully validated in any product of concern.

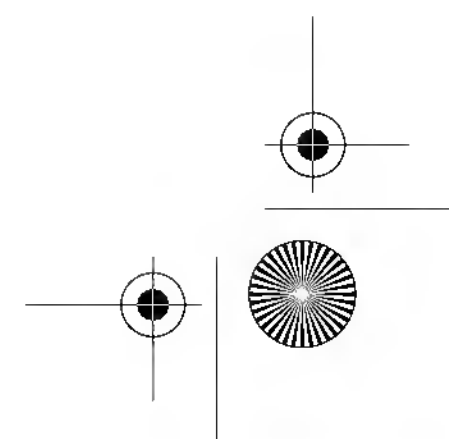
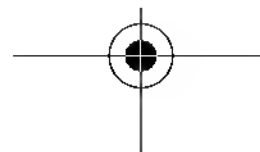
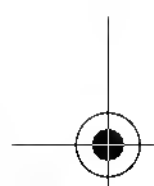
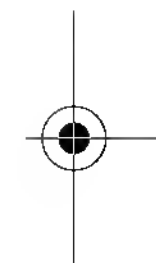
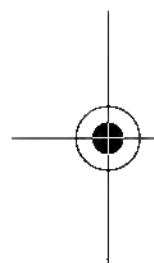
REFERENCES

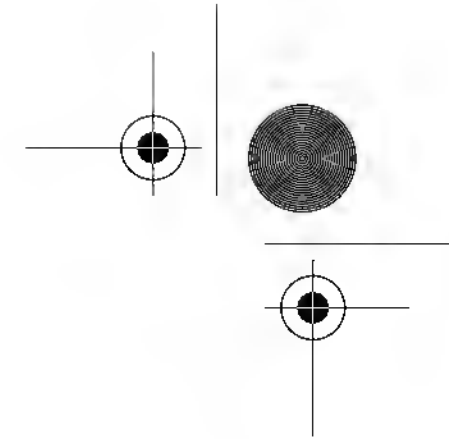
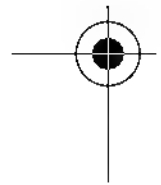
1. Abdulraouf, U.M., L.R. Beuchat, and M.S. Ammar. 1993. Survival and growth of *Escherichia coli* O157-H7 on salad vegetables. *Appl. Environ. Microbiol.* 59:1999–2006.
2. Abiss, J.S. 1983. Injury and resuscitation of microbes with reference to food microbiology. *Irish J. Food Sci. Technol.* 7:69–81.
3. Ahamad, N. and E.H. Marth. 1989. Behavior of *Listeria monocytogenes* at 7, 13, 21, and 35°C in tryptose broth acidified with acetic, citric, or lactic-acid. *J. Food Prot.* 52:688–695.
4. Alexandrou, O., C.D.W. Blackburn, and M.R. Adams. 1995. Capacitance measurement to assess acid-induced injury to *S. enteritidis* PT4. *Int. J. Food Microbiol.* 27(1):27–36.
5. Andrews, W.H. 1986. Resuscitation of injured *Salmonella* spp. and coliforms from foods. *J. Food Prot.* 49:62–75.
6. Augustin, J.C., A. Brouillaud-Delattre, L. Rosso, and V. Carlier. 2000. Significance of inoculum size in the lag time of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 66:1706–1710.
7. Augustin, J.C. and V. Carlier. 2000. Mathematical modelling of the growth rate and lag time for *Listeria monocytogenes*. *Int. J. Food Microbiol.* 56:29–51.
8. Augustin, J.C. and V. Carlier. 2000. Modelling the growth rate of *Listeria monocytogenes* with a multiplicative type model including interactions between environmental factors. *Int. J. Food Microbiol.* 56:53–70.
9. Augustin, J.C., V. Carlier, and J. Rozier. 1998. Mathematical modelling of the heat resistance of *Listeria monocytogenes*. *J. Appl. Microbiol.* 84:185–191.
10. Augustin, J.C., L. Rosso, and V. Carlier. 2000. A model describing the effect of temperature history on lag time for *Listeria monocytogenes*. *Int. J. Food Microbiol.* 57:169–181.
11. Baird-Parker, A.C. 1980. Organic acids. In *Microbial Ecology of Foods, Vol. 1: Factors Affecting Life and Death of Micro-Organisms*. Academic Press, New York, pp. 126–135. International Commission on Microbiological Specifications for Foods.



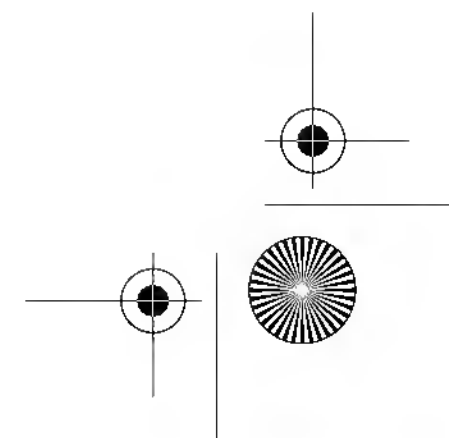
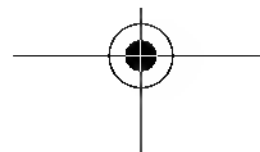
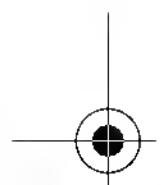
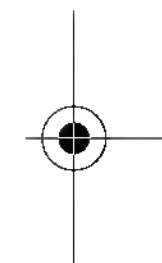
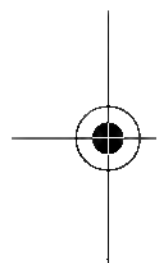


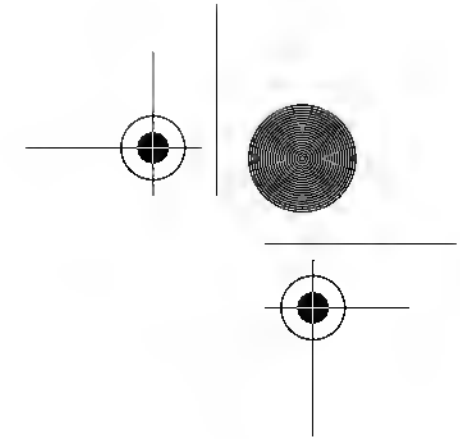
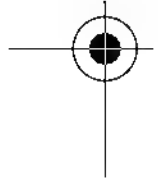
12. Baird-Parker, A.C. and B. Freame. 1967. Combined effect of water activity, pH and temperature on the growth of *Clostridium botulinum* from spore and vegetative cell inocula. *J. Appl. Bacteriol.* 30:420–429.
13. Baranyi, J. and T.A. Roberts. 1994. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.*, 23:277–294.
14. Baranyi, J. and T.A. Roberts. 1995. Mathematics of predictive food microbiology. *Int. J. Food Microbiol.* 26:199–218.
15. Barrell, R.A.E. 1988. The survival and recovery of *Salmonella typhimurium* phage type U285 in frozen meats and tryptone soya yeast extract broth. *Int. J. Food Microbiol.* 6:309–316.
16. Begot, C., I. Lebert, and A. Lebert. 1997. Variability of the response of 66 *Listeria monocytogenes* and *Listeria innocua* strains to different growth conditions. *Food Microbiol.* 14:403–412.
17. Bellara, S.R., P.J. Fryer, C.M. McFarlane, C.R. Thomas, P.M. Hocking, and B.M. Mackey. 1999. Visualization and modelling of the thermal inactivation of bacteria in a model food. *Appl. Environ. Microbiol.* 65:3095–3099.
18. Bello, J. and M.A. Sanchezfuertes. 1995. Application of a mathematical model for the inhibition of Enterobacteriaceae and clostridia during a sausage curing process. *J. Food Prot.* 58:1345–1350.
19. Bello, J. and M.A. Sanchezfuertes. 1995. Application of a mathematical model to describe the behaviour of the *Lactobacillus* spp. during the ripening of a Spanish dry fermented sausage (chorizo). *Int. J. Food Microbiol.* 27:215–227.
20. Benito, A., G. Ventoura, M. Casadei, T. Robinson, and B. Mackey. 1999. Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses. *Appl. Environ. Microbiol.* 65:1564–1569.
21. Bennik, M.H.J., E.J. Smid, F.M. Rombouts, and L.G.M. Gorris. 1995. Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiol.* 12:509–519.
22. Bhaduri, S., C.O. Turnerjones, R.L. Buchanan, and J.G. Phillips. 1994. Response surface model of the effect of pH, sodium chloride and sodium nitrite on growth of *Yersinia enterocolitica* at low temperatures. *Int. J. Food Microbiol.* 23:333–343.
23. Blackburn, C.D., L.M. Curtis, L. Humpheson, C. Billon, and P.J. McClure. 1997. Development of thermal inactivation models for *Salmonella enteritidis* and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. *Int. J. Food Microbiol.* 38:31–44.
24. Bouttefroy, A., M. Linder, and J.B. Milliere. 2000. Predictive models of the combined effects of curvaticin 13, NaCl and on the behaviour of *Listeria monocytogenes* ATCC 15313 in broth. *J. Appl. Microbiol.* 88; 919–929.
25. Bovill, R., J. Bew, N. Cook, M. D'Agostino, N. Wilkinson, and J. Baranyi. 2000. Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature. *Int. J. Food Microbiol.* 59:157–165.
26. Bovill, R.A., J.A. Shallcross, and B.M. Mackey. 1994. Comparison of the fluorescent redox dye 5-cyano-2,3-ditolyltetrazolium chloride with *p*-iodonitrotetrazolium violet to detect metabolic activity in heat-stressed *Listeria monocytogenes* cells. *J. Appl. Bacteriol.* 77(4):353–358.
27. Brocklehurst, T.F. 1994. Delicatessen salads, and chilled prepared fruit and vegetable products. In *Evaluation of Shelf Life of Foods—Principles and Practice*, Man, D. and Jones, A. (Eds.). Chapman & Hall, Glasgow, pp. 87–126.



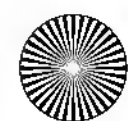
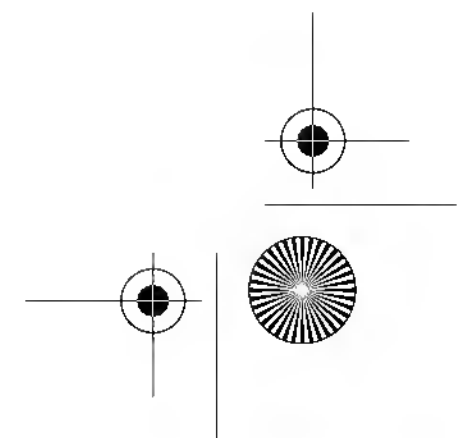
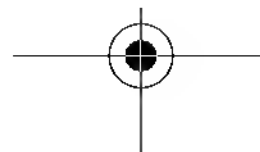
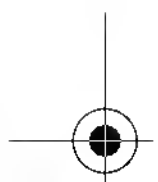
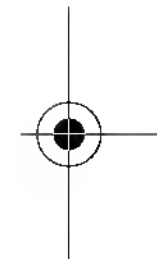
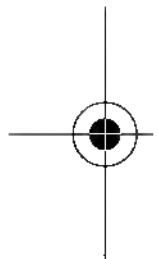


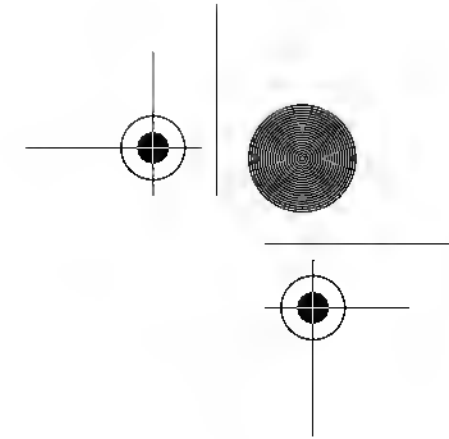
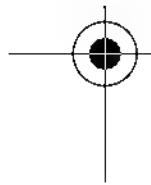
28. Brocklehurst, T.F., G.A. Mitchell, Y.P. Ridge, R. Seale, and A.C. Smith. 1995. The effect of transient temperatures on the growth of *Salmonella typhimurium* LT2 in gelatin gel. *Int. J. Food Microbiol.* 27:45–60.
29. Brocklehurst, T.F., G.A. Mitchell, and A.C. Smith. 1997. A model experimental gel-surface for the growth of bacteria on foods. *Food Microbiol.* 14:303–311.
30. Brocklehurst, T.F., M.L. Parker, P.A. Gunning, H.P. Coleman, and M.M. Robins. 1995. Growth of food-borne pathogenic bacteria in oil-in-water emulsions. II. Effect of emulsion structure on growth parameters and form of growth. *J. Appl. Bacteriol.* 78:609–615.
31. Brocklehurst, T.F., M.L. Parker, P.A. Gunning, and M.M. Robins. 1993. Microbiology of emulsions: physicochemical aspects. *Lipid Technol.* July/August: 83–88.
32. Brocklehurst, T.F. and P.D.G. Wilson. 2000. The role of lipids in controlling microbial growth. *Grasas y Aceitas* 51:66–73.
33. Brouillaud-Delatre, A., M. Maire, C. Collette, C. Mattei, and C. Lahellec. 1997. Predictive microbiology of dairy products: influence of biological factors affecting growth of *Listeria monocytogenes*. *J. AOAC Int.* 80:913–919.
34. Brul, S. and P. Coote. 1999. Preservative agents in foods: mode of action and microbial resistance mechanisms. *Int. J. Food Microbiol.* 50:1–17.
35. Buchanan, R.L. and L.K. Bagi. 1997. Microbial competition: effect of culture conditions on the suppression of *Listeria monocytogenes* Scott A by *Carnobacterium piscicola*. *J. Food Prot.* 60:254–261.
36. Buchanan, R.L. and L.K. Bagi. 1997. Effect of water activity and humectant identity on the growth kinetics of *Escherichia coli* O157:H7. *Food Microbiol.* 14:413–423.
37. Buchanan, R.L., L.K. Bagi, R.V. Goins, and J.G. Phillips. 1993. Response-surface models for the growth-kinetics of *Escherichia coli* O157H7. *Food Microbiol.* 10:303–315.
38. Buchanan, R.L. and M.H. Golden. 1998. Interactions between pH and malic acid concentration on the inactivation of *Listeria monocytogenes*. *J. Food Safety* 18:37–48.
39. Buchanan, R.L., M.H. Golden, and J.G. Phillips. 1997. Expanded models for the non-thermal inactivation of *Listeria monocytogenes*. *J. Appl. Microbiol.* 82:567–577.
40. Buchanan, R.L., M.H. Golden, and R.C. Whiting. 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. *J. Food Prot.* 56:474–478 and 484.
41. Buchanan, R.L. and L.A. Klawitter. 1992. The effect of incubation-temperature, initial pH, and sodium chloride on the growth-kinetics of *Escherichia coli* O157-H7. *Food Microbiol.* 9:185–196.
42. Buchanan, R.L., H.G. Stahl, and R.C. Whiting. 1989. Effects and interactions of temperature, pH, atmosphere, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. *J. Food Prot.* 52:844–851.
43. Buchanan, R.L. and R.C. Whiting. 1996. Risk assessment and predictive microbiology. *J. Food Prot.* 31–36.
44. Cabo, M.L., M.A. Murado, M.P. Gonzalez, and L. Pastoriza. 2000. Dose–response relationships. A model for describing interactions, and its application to the combined effect of nisin and lactic acid on *Leuconostoc mesenteroides*. *J. Appl. Microbiol.* 88:756–763.
45. Casadei, M.A., R. Ingram, E. Hitchings, J. Archer, and J.E. Gaze. 2001. Heat resistance of *Bacillus cereus*, *Salmonella typhimurium* and *Lactobacillus delbrueckii* in relation to pH and ethanol. *Int. J. Food Microbiol.* 63:125–134.



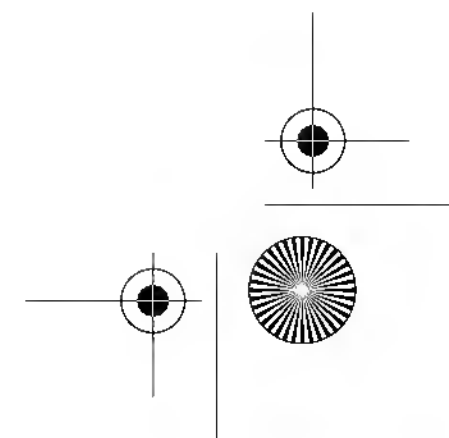
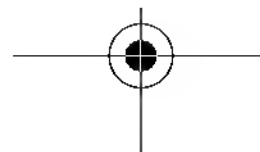
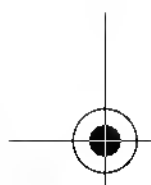
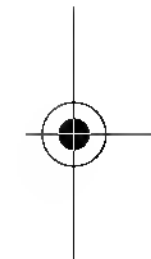
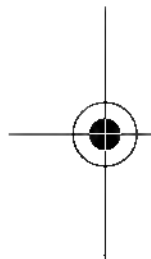


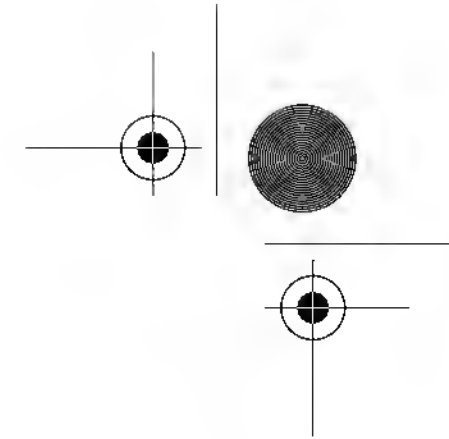
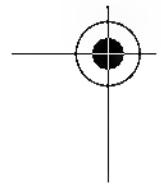
46. Chea, F.P., Y.H. Chen, T.J. Montville, and D.W. Schaffner. 2000. Modeling the germination kinetics of *Clostridium botulinum* 56A spores as affected by temperature, pH, and sodium chloride. *J. Food Prot.* 63:1071–1079.
47. Cheroutre-Vialette, M. and A. Lebert. 2000. Growth of *Listeria monocytogenes* as a function of dynamic environment at 10°C and accuracy of growth predictions with available models. *Food Microbiol.* 17:83–92.
48. Chhabra, A.T., W.H. Carter, R.H. Linton, and M.A. Cousin. 1999. A predictive model to determine the effects of pH, milk fat, and temperature on thermal inactivation of *Listeria monocytogenes*. *J. Food Prot.* 62:1143–1149.
49. Christian, J.H.B. 1980. Reduced water activity. In *Microbial Ecology of Foods, Vol. 1: Factors Affecting Life and Death of Micro-Organisms*. Academic Press, New York, pp. 70–91.
50. Clayson, D.H.F. and R.M. Blood. 1957. Food perishability: the determination of the vulnerability of food surfaces to bacterial infection. *J. Sci. Food Agri.* 8:404–414.
51. Conner, D.E., V.N. Scott, and D.T. Bernard. 1990. Growth, inhibition, and survival of *Listeria monocytogenes* as affected by acidic conditions. *J. Food Prot.* 53:652–655.
52. Conner, D.E. and J.S. Kotrola. 1995. Growth and survival of *Escherichia coli* O157-H7 under acidic conditions. *Appl. Environ. Microbiol.* 61:382–385.
53. Cooper, A.L., A.C.R. Dean, and C. Hinshelwood. 1968. Factors affecting the growth of bacterial colonies on agar plates. *Proc. Royal Soc. B.* 171:175–199.
54. Curtis, L.M., M. Patrick, and C.D. Blackburn. 1995. Survival of *Campylobacter jejuni* in foods and comparison with a predictive model. *Lett. Appl. Microbiol.* 21:194–197.
55. Daughtry, G.J., K.R. Davey, and K.D. King. 1997. Temperature dependence of growth kinetics of food bacteria. *Food Microbiol.* 14:21–30.
56. Davis, M. J., P.J. Coote, and C.P. O’Byrne. 1996. Acid tolerance in *Listeria monocytogenes*: the adaptive acid tolerance response (ATR) and growth-phase-dependent acid resistance. *Microbiology (UK)* 142:2975–2982.
57. Delignette-muller, M.L., L. Rosso, and J.P. Flandrois. 1995. Accuracy of microbial growth predictions with square root and polynomial models. *Int. J. Food Microbiol.* 27:139–146.
58. Devlieghere, F., B. VanBelle, and J. Debevere. 1999. Shelf life of modified atmosphere packed cooked meat products: a predictive model. *Int. J. Food Microbiol.* 46:57–70.
59. Dilworth, M. J., A.R. Glenn, W.N. Konings, I.R. Booth, R.K. Poole, T.A. Krulwich, R.J. Rowbury, J.B. Stock, J.L. Slonczewski, G.M. Cook, E. Padan, H. Kobayashi, G.N. Bennett, A. Matin, and V. Skulachev. 1999. Problems of adverse pH and bacterial strategies to combat it. *Bacterial Response to pH*, Novartis Foundation Symposium 221. John Wiley & Sons, New York. 221:4–18.
60. Dodd, C. 1990. Detection of microbial growth in food by cryosectioning and light microscopy. *Food Sci. Tech. Today* 4(3):180–182.
61. Dodd, C. and W.M. Waites. 1991. The use of toluidine blue for *in situ* detection of micro-organisms in foods. *Lett. Appl. Microbiol.* 13:220–223.
62. Duffy, G., A. Ellison, W. Anderson, M.B. Cole, and G.S.A.B. Stewart. 1995. Use of bioluminescence to model the thermal inactivation of *Salmonella typhimurium* in the presence of a competitive microflora. *Appl. Environ. Microbiol.* 61:3463–3465.
63. Duffy, L.L., P.B. Vanderlinde, and F.H. Grau. 1994. Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, a_w , nitrite and ascorbate. *Int. J. Food Microbiol.* 23:377–390.
64. Eifert, J.D., C.R. Hackney, M.D. Pierson, S.E. Duncan, and W.N. Eigel. 1997. Acetic, lactic, and hydrochloric acid effects on *Staphylococcus aureus* 196E growth based on a predictive model. *J. Food Sci.* 62:174–178.



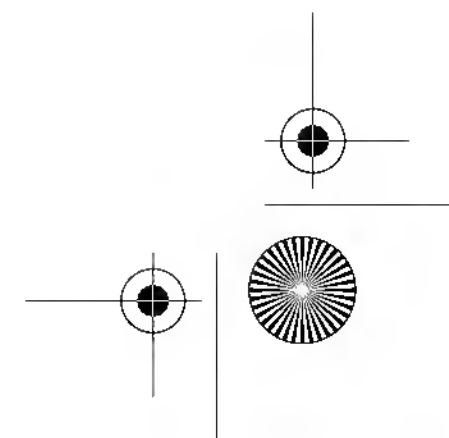
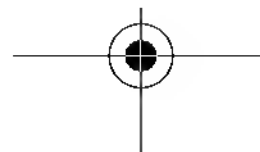
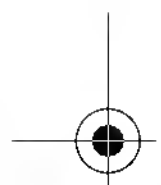
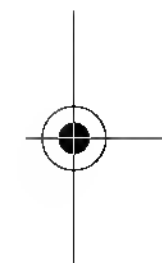
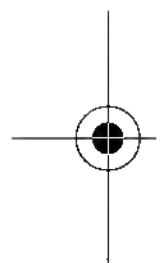


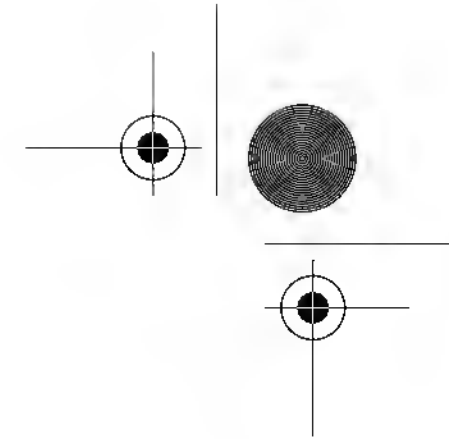
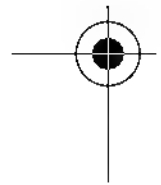
65. Eklund, T. 1983. The antimicrobial effects of dissociated and undissociated sorbic acid at different pH levels. *J. Appl. Bacteriol.* 54:383–389.
66. Ellajosyula, K.R., S. Doores, E.W. Mills, R.A. Wilson, R.C. Anantheswaran, and S.J. Knabel. 1998. Destruction of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in Lebanon bologna by interaction of fermentation pH, heating temperature, and time. *J. Food Prot.* 61:152–157.
67. Ellison, A., S.F. Perry, and G.S.A.B. Stewart. 1991. Bioluminescence as a real-time monitor of injury and recovery in *Salmonella typhimurium*. *Int. J. Food Microbiol.* 12:323–332.
68. Entani, E., M. Asai, S. Tsujihata, Y. Tsukamoto, and M. Ohta. 1998. Antibacterial action of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7. *J. Food Prot.* 61:953–959.
69. Farber, J.M., R.C. McKellar, and W.H. Ross. 1995. Modelling the effects of various parameters on the growth of *Listeria monocytogenes* on liver pâté. *Food Microbiol.* 12:447–453.
70. Fernandez, A., M.J. Ocio, P.S. Fernandez, and A. Martinez. 2001. Effect of heat activation and inactivation conditions on germination and thermal resistance parameters of *Bacillus cereus* spores. *Int. J. Food Microbiol.* 63:257–264.
71. Fernandez, P.S., S.M. George, C.C. Sills, and M.W. Peck. 1997. Predictive model of the effect of CO₂, pH, temperature and NaCl on the growth of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 37:37–45.
72. George, S.M., B.M. Lund, and T.F. Brocklehurst. 1988. The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. *Lett. Appl. Microbiol.* 6:153–156.
73. Gill, C.O., G.G. Greer, and B.D. Dilts. 1998. Predicting the growth of *Escherichia coli* on displayed pork. *Food Microbiol.* 15:235–242.
74. Glass, K.A., J.M. Loeffelholz, J.P. Ford, and M.P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. *Appl. Environ. Microbiol.* 58:2513–2516.
75. Gomez, R.F. and Herrero, A.A. 1983. Chemical preservation of foods. In *Food Microbiology*, Rose, A.H. (Ed.). Academic Press, London, pp. 78–116.
76. Graham, A.F., D.R. Mason, and M.W. Peck. 1996. A predictive model of the effect of temperature, pH and sodium chloride on growth from spores of non-proteolytic *Clostridium botulinum*. *Int. J. Food Microbiol.* 31:69–85.
77. Hills, B.P., C.E. Manning, Y.P. Ridge, and T.F. Brocklehurst. 1996. NMR water relaxation, water activity and bacterial survival in porous media *J. Sci. Food Agric.* 71:185–194.
78. Hills, B.P., C.E. Manning, Y.P. Ridge, and T.F. Brocklehurst. 1997. Water availability and the survival of *Salmonella typhimurium* in porous systems. *Int. J. Food Microbiol.* 36:187–198.
79. Hills, B.P. and K.M. Wright. 1994. A new model for bacterial growth in heterogeneous systems. *J. Theor. Biol.* 168:31–41.
80. Hoffmans, C.M., D.Y.C. Fung, and C.L. Kastner. 1997. Methods and resuscitation environments for the recovery of heat-injured *Listeria monocytogenes*: a review. *J. Rapid Meth. Automat. Microbiol.* 5(4):249–268.
81. Houtsma, P.C., M.L. Kant-Muermans, F.M. Rombouts, and M.H. Zwietering. 1996. Model for the combined effects of temperature, pH, and sodium lactate on growth rates of *Listeria innocua* in broth and Bologna-type sausages. *Appl. Environ. Microbiol.* 62:1616–1622.



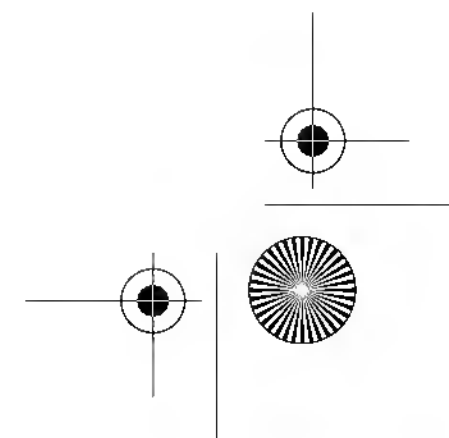
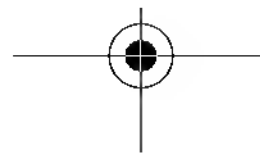
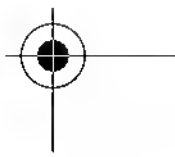
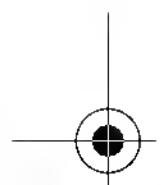
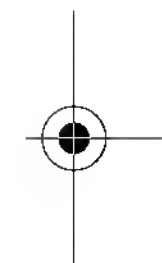
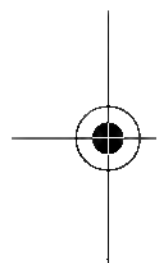


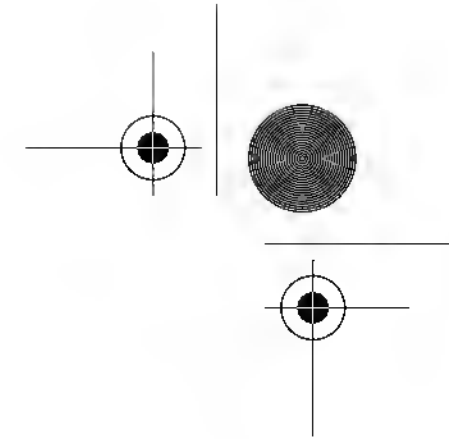
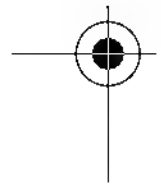
82. Hurst, A. 1977. Bacterial injury: a review. *Can. J. Microbiol.* 23:935–944.
83. International Commission on Microbiological Specifications for Foods. 1996. *Microorganisms in Foods, Vol. 5: Microbiological Specifications of Food Pathogens*. Blackie Academic and Professional, London.
84. Johnson, S., E. Parsons, S. Stringer, T.F. Brocklehurst, C. Dodd, M. Morgan, and W. Waites. 1996. Multixenic growth of micro-organisms in food. *Food Sci. Tech. Today* 12(1):53–56.
85. Jeyamkondan, S., D.S. Jayas, and R.A. Holley. 2001. Microbial growth modeling with artificial neural networks. *Int. J. Food Microbiol.* 64:343–354.
86. Juneja, V.K. and B.S. Eblen. 1999. Predictive thermal inactivation model for *Listeria monocytogenes* with temperature, pH, NaCl, and sodium pyrophosphate as controlling factors. *J. Food Prot.* 62:986–993.
87. Juneja, V.K., B.S. Marmer, and B.S. Eblen. 1999. Predictive model for the combined effect of temperature, pH, sodium chloride, and sodium pyrophosphate on the heat resistance of *Escherichia coli* O157:H7. *J. Food Safety* 19:147–160.
88. Katsaras, K. and L. Leistner. 1991. Distribution and development of bacterial colonies in fermented sausages. *Biofouling* 5:1145–124.
89. Koutsoumanis, K., K. Lambropoulou, and G.J.E. Nychas. 1999. A predictive model for the non-thermal inactivation of *Salmonella enteritidis* in a food model system supplemented with a natural antimicrobial. *Int. J. Food Microbiol.* 49:63–74.
90. Koutsoumanis, K. and G.J.E. Nychas. 2000. Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. *Int. J. Food Microbiol.* 60:171–184.
91. Koutsoumanis, K.P., P.S. Taoukis, E.H. Drosinos, and G.J.E. Nychas. 2000. Applicability of an Arrhenius model for the combined effect of temperature and CO₂ packaging on the spoilage microflora of fish. *Appl. Environ. Microbiol.* 66:3528–3534.
92. Kroll, R.G. and R.A. Patchett. 1992. Induced acid tolerance in *Listeria monocytogenes*. *Lett. Appl. Microbiol.* 14:224–227.
93. Labuza, T.P. and Bell, L.N. 1984. *Moisture Sorption. Practical Isotherm Measurement and Use*, 2nd ed. American Association of Cereal Chemists, St. Paul, MN.
94. Lebert, I., V. Robles-Olvera, and A. Lebert. 2000. Application of polynomial models to predict growth of mixed cultures of *Pseudomonas* spp. and *Listeria* in meat. *Int. J. Food Microbiol.* 61:27–39.
95. Lee, I.S., J.L. Slonczewski, and J.W. Foster. 1994. A low-pH-inducible, stationary-phase acid tolerance response in *Salmonella typhimurium*. *J. Bacteriol.* 176:1422–1426.
96. Leguerinel, I. and P. Mafart. 2001. Modelling the influence of pH and organic acid types on thermal inactivation of *Bacillus cereus* spores. *Int. J. Food Microbiol.* 63:29–34.
97. Leistner, L. 1992. Food preservation by combined methods. *Food Res. Int.* 25:151–158.
98. Leistner, L. and L.G.M. Gorris. 1995. Food preservation by hurdle technology. *Trends Food Sci. Tech.* 6:41–46.
99. Le Marc, Y., V. Huchet, C.M. Bourgeois, J.P. Guyonnet, P. Mafart, and D. Thuault. 2002. Modelling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. *Int. J. Food Microbiol.* 73:219–237.
100. Little, C.L., M.R. Adams, W.A. Anderson, and M.B. Cole. 1994. Application of a log-logistic model to describe the survival of *Yersinia enterocolitica* at sub-optimal pH and temperature. *Int. J. Food Microbiol.* 22:63–71.
101. Llaudes, M.K., L.H. Zhao, S. Duffy, and D.W. Schaffner. 2001. Simulation and modelling of the effect of small inoculum size on time to spoilage by *Bacillus stearothermophilus*. *Food Microbiol.* 18:395–405.



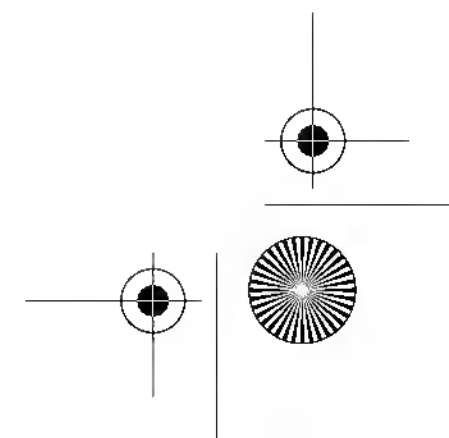
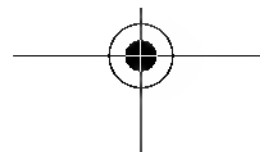
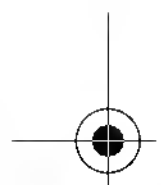
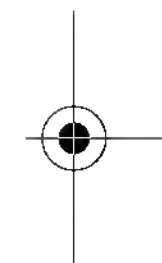
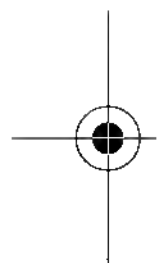


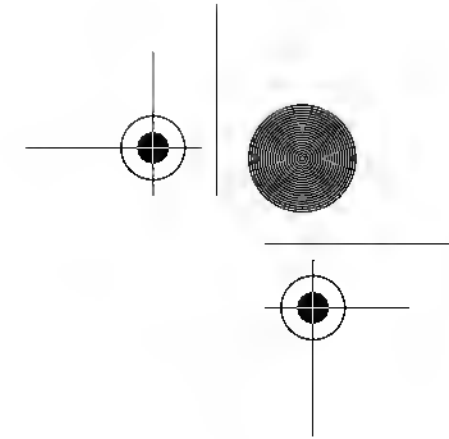
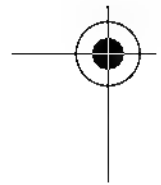
102. Mackey, B.M. and C.M. Derrick. 1982. The effects of sub-lethal injury by heating, freezing, drying and gamma-radiation on the duration of the lag phase of *Salmonella typhimurium*. *J. Appl. Bacteriol.* 53:243–251.
103. Mackey, B.M., E. Boogard, C.M. Hayes, and J. Baranyi. 1994. Recovery of heat-injured *Listeria monocytogenes*. *Int. J. Food Microbiol.* 22(4):227–237.
104. Malakar, P., T.F. Brocklehurst, A.R. Mackie, P.D.G. Wilson, M.H. Zwietering, and K. Van't Riet. 2000. Microgradients in bacterial colonies: use of fluorescent ratio imaging, a non-invasive technique. *Int. J. Food Microbiol.* 56:71–80.
105. Malakar, P.K., D.E. Martens, M.H. Zwietering, C. Beal, and K. Van't Riet. 1999. Modelling the interactions between *Lactobacillus curvatus* and *Enterobacter cloacae*. II. Mixed cultures and shelf life predictions. *Int. J. Food Microbiol.* 51:67–79.
106. Manas, P., R. Pagan, I. Leguerinel, S. Condon, P. Mafart, and F. Sala. 2001. Effect of sodium chloride concentration on the heat resistance and recovery of *Salmonella typhimurium*. *Int. J. Food Microbiol.* 63:209–216.
107. Martens, D.E., C. Beal, P.K. Malakar, M.H. Zwietering, and K. Van't Riet. 1999. Modelling the interactions between *Lactobacillus curvatus* and *Enterobacter cloacae*. I. Individual growth kinetics. *Int. J. Food Microbiol.* 51:53–65.
108. Marth, E.H. 1998. Extended shelf life refrigerated foods: microbiological quality and safety. *Food Tech.* 52:57–62.
109. Mattila, T. and A.J. Frost. 1988. The growth of potential food poisoning organisms on chicken and pork muscle surfaces. *J. Appl. Bacteriol.* 65:455–461.
110. Mattila, T. and A.J. Frost. 1988. Colonization of beef and chicken muscle surfaces by *Escherichia coli*. *Food Microbiol.* 5:219–230.
111. McClure, P.J., A.L. Beaumont, J.P. Sutherland, and T.A. Roberts. 1997. Predictive modelling of growth of *Listeria monocytogenes*: the effects on growth of NaCl, pH, storage temperature and NaNO₂. *Int. J. Food Microbiol.* 34:221–232.
112. McClure, P.J., M.B. Cole, and K.W. Davies. 1994. An example of the stages in the development of a predictive mathematical model for microbial growth: the effects of NaCl, pH and temperature on the growth of *Aeromonas hydrophila*. *Int. J. Food Microbiol.* 23:359–375.
113. McDonald, K. and D.W. Sun. 1999. Predictive food microbiology for the meat industry: a review. *Int. J. Food Microbiol.* 52:1–27.
114. McElroy, D.M., L.A. Jaykus, and P.M. Foegeding. 2000. Validation and analysis of modeled predictions of growth of *Bacillus cereus* spores in boiled rice. *J. Food Prot.* 63:268–272.
115. McKay, A.L. and A.C. Peters. 1995. The effect of sodium chloride concentration and pH on the growth of *Salmonella typhimurium* colonies on solid medium. *J. Appl. Bacteriol.* 79:353–359.
116. McKellar, R.C. and X.W. Lu. 2001. A probability of growth model for *Escherichia coli* O157:H7 as a function of temperature, pH, acetic acid, and salt. *J. Food Prot.* 64:1922–1928.
117. McKellar, R.C., G. Butler, and K. Stanich. 1997. Modelling the influence of temperature on the recovery of *Listeria monocytogenes* from heat injury. *Food Microbiol.* 14:617–625.
118. McKellar, R.C., R. Moir, and M. Kaleb. 1994. Factors influencing the survival and growth of *Listeria monocytogenes* on the surface of Canadian retail wieners. *J. Food Prot.* 57:387–392.
119. McMeekin, T.A., J.N. Olley, T. Ross, and D.A. Ratkowsky. 1993. *Predictive Microbiology: Theory and Application*. Wiley, New York.



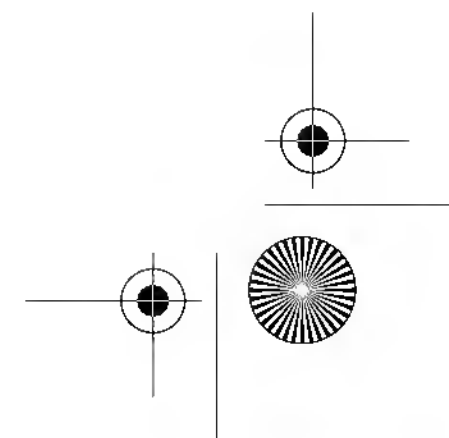
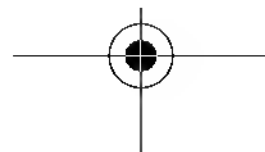
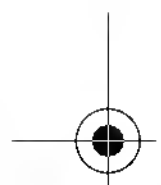
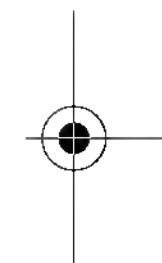
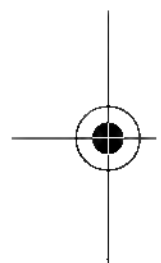


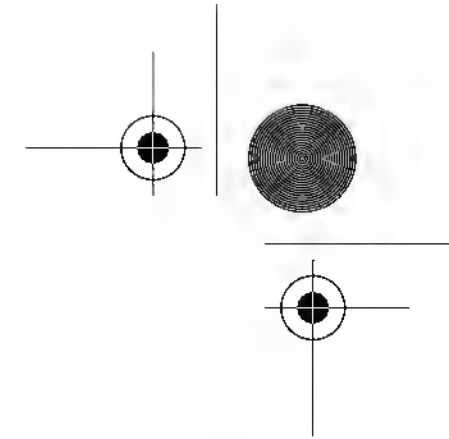
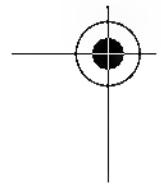
120. McMeekin, T.A., K. Presser, D. Ratkowsky, T. Ross, M. Salter, and S. Tienungoon. 2000. Quantifying the hurdle concept by modelling the bacterial growth/no growth interface. *Int. J. Food Microbiol.* 55:93–98.
121. Membre, J.M., V. Majchrzak, and I. Jolly. 1997. Effects of temperature, pH, glucose, and citric acid on the inactivation of *Salmonella typhimurium* in reduced calorie mayonnaise. *J. Food Prot.* 60:1497–1501.
122. Miles, D.W. and T. Ross. 1999. Identifying and quantifying risks in the food production chain. *Food Aust.* 51:298–303.
123. Miles, D.W., T. Ross, J. Olley, and T.A. McMeekin. 1997. Development and evaluation of a predictive model for the effect of temperature and water activity on the growth rate of *Vibrio parahaemolyticus*. *Int. J. Food Microbiol.* 38:133–142.
124. Mitchell, A.J. and J.W.T. Wimpenny. 1997. The effects of agar concentration on the growth and morphology of submerged colonies of motile and non-motile bacteria. *J. Appl. Bacteriol.* 83:76–84.
125. Mitchell, G.A., T.F. Brocklehurst, R. Parker, and A.C. Smith. 1994. The effect of transient temperatures on the growth of *Salmonella typhimurium* LT2. I. Cycling within the growth region. *J. Appl. Bacteriol.* 77:113–119.
126. Mitchell, G.A., T.F. Brocklehurst, R. Parker, and A.C. Smith. 1995. The effect of transient temperatures on the growth of *Salmonella typhimurium* LT2. II. Excursions outside the growth region. *J. Appl. Bacteriol.* 79:128–134.
127. Mossel, D.A.A., J.E.L. Corry, C.B. Struijk, and R.M. Baird. 1995. *Essentials of the Microbiology of Foods: A Textbook for Advanced Studies*. Wiley, Chichester, UK.
128. Morinigo, M.A., R. Cornax, D. Castro, E. Martinez-Manzanares, and J.J. Borrego. 1990. Viability of *Salmonella* spp. and indicator micro-organisms in seawater using membrane diffusion chambers. *Antonie van Leeuwenhoek* 57(2):109–118.
129. Murphy, P.M., M.C. Rea, and D. Harrington. 1996. Development of a predictive model for growth of *Listeria monocytogenes* in a skim milk medium and validation studies in a range of dairy products. *J. Appl. Bacteriol.* 80:557–564.
130. Nerbrink, E., E. Borch, H. Blom, and T. Nesbakken. 1999. A model based on absorbance data on the growth rate of *Listeria monocytogenes* and including the effects of pH, NaCl, Na-lactate and Na-acetate. *Int. J. Food Microbiol.* 47:99–109.
131. Neumeyer, K., T. Ross, and T.A. McMeekin. 1997. Development of a predictive model to describe the effects of temperature and water activity on the growth of spoilage pseudomonads. *Int. J. Food Microbiol.* 38:45–54.
132. Nicolai, B.M., J.F. van Impe, B. Verlinden, T. Martens, J. Vandewalle, and J. De Baerdemaeker. 1993. Predictive modelling of surface growth of lactic acid bacteria in vacuum-packed meat. *Food Microbiol.* 10:229–238.
133. Nyati, H. 2000. Survival characteristics and the applicability of predictive mathematical modelling to *Listeria monocytogenes* growth in sous vide products. *Int. J. Food Microbiol.* 56:123–132.
134. O'Driscoll, B., C.G.M. Gahan, and C. Hill. 1996. Adaptive acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. *Appl. Environ. Microbiol.* 62:1693–1698.
135. Oscar, T.P. 1999. Response surface models for effects of temperature and previous growth sodium chloride on growth kinetics of *Salmonella typhimurium* on cooked chicken breast. *J. Food Prot.* 62:1470–1474.
136. Oscar, T.P. 1999. Response surface models for effects of temperature and previous temperature on lag time and specific growth rate of *Salmonella typhimurium* on cooked ground chicken breast. *J. Food Prot.* 62:1111–1114.



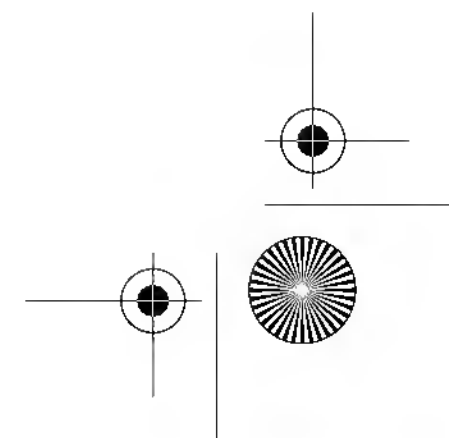
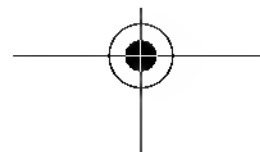
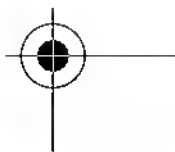
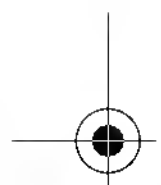
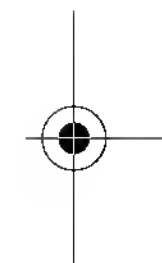
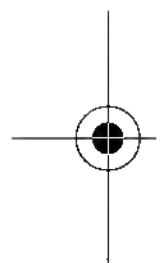


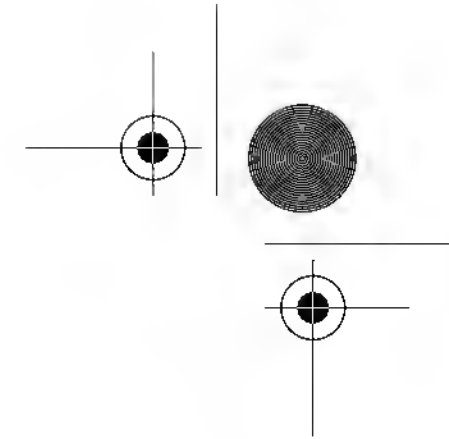
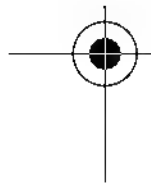
137. Oscar, T.P. 1999. Response surface models for effects of temperature, pH and previous growth pH on growth kinetics of *Salmonella typhimurium* in brain heart infusion broth. *J. Food Prot.* 62:106–111.
138. Parente, E., M.A. Giglio, A. Ricciardi, and F. Clementi. 1998. The combined effect of nisin, leucocin F10, pH, NaCl and EDTA on the survival of *Listeria monocytogenes* in broth. *Int. J. Food Microbiol.* 40:65–75.
139. Parker, M.L., T.F. Brocklehurst, P.A. Gunning, H.P. Coleman, and M.M. Robins. 1995. Growth of food-borne pathogenic bacteria in oil-in-water emulsions. I. Methods for investigating the form of growth of bacteria in model oil-in-water emulsions and dairy cream. *J. Appl. Bacteriol.* 78:601–608.
140. Parker, M.L., P.A. Gunning, A.C. Macedo, F.X. Malcata, and T.F. Brocklehurst. 1998. The microstructure and distribution of micro-organisms within mature Serra cheese. *J. Appl. Microbiol.* 84:523–530.
141. Peters, A.C., J.W.T. Wimpenny, and J.P. Coombs. 1987. Oxygen profiles in, and in the agar beneath, colonies of *Bacillus cereus*, *Staphylococcus albus* and *Escherichia coli*. *J. Gen. Microbiol.* 133:1257–1263.
142. Pin, C. and J. Baranyi. 1998. Predictive models as means to quantify the interactions of spoilage organisms. *Int. J. Food Microbiol.* 41:59–72.
143. Pin, C., J. Baranyi, and G. de Fernando. 2000. Predictive model for the growth of *Yersinia enterocolitica* under modified atmospheres. *J. Appl. Microbiol.* 88:521–530.
144. Pin, C., J.P. Sutherland, and J. Baranyi. 1999. Validating predictive models of food spoilage organisms. *J. Appl. Microbiol.* 87:491–499.
145. Piyasena, P., S. Liou, and R.C. McKellar. 1998. Predictive modelling of inactivation of *Listeria* spp. in bovine milk during HTST pasteurization. *Int. J. Food Microbiol.* 39:167–173.
146. Potts, M. 1994. Desiccation tolerance of prokaryotes. *Microbiol. Rev.* 58:755–805.
147. Presser, K.A., T. Ross, and D.A. Ratkowsky. 1998. Modelling the growth limits (growth no growth interface) of *Escherichia coli* as a function of temperature, pH, lactic acid concentration, and water activity. *Appl. Environ. Microbiol.* 64:1773–1779.
148. Ratkowsky, D.A., R.K. Lowry, T.A. McMeekin, A.N. Stokes, and R.E. Chandle. 1983. Model for bacterial culture-growth rate throughout the entire biokinetic temperature-range. *J. Bacteriol.* 154(3):1222–1226.
149. Ratkowsky, D.A. and T. Ross. 1995. Modelling the bacterial growth/no growth interface. *Lett. Appl. Microbiol.* 20:29–33.
150. Ray, B. 1979. Methods to detect stressed micro-organisms. *J. Food Prot.* 42:346–355.
151. Riordan, D.C., G. Duffy, J.J. Sheridan, B.S. Eblen, R.C. Whiting, I.S. Blair, and D.A. McDowell. 1998. Survival of *Escherichia coli* O157:H7 during the manufacture of pepperoni. *J. Food Prot.* 61:146–151.
152. Robins, M.M. and P.D.G. Wilson. 1994. Food structure and microbial growth. *Trends Food Sci. Tech.* 5:289–293.
153. Robins, M.M., T.F. Brocklehurst, and P.D.G. Wilson. 1994. Food structure and the growth of pathogenic bacteria. *Food Technol. Int. (Eur.)* 31–36.
154. Robinson, R.A. and R.H. Stokes. 1959. *Electrolyte Solutions*. Butterworths, London.
155. Robinson, T.P., J.W.T. Wimpenny, and R.G. Earnshaw. 1991. pH gradients through colonies of *Bacillus cereus* and the surrounding agar. *J. Gen. Microbiol.* 137:2885–2889.
156. Rocelle, M., S. Clavero, and L.R. Beuchat. 1996. Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH, water activity, and temperature and suitability of media for its recovery. *Appl. Environ. Microbiol.* 62:8–2740.



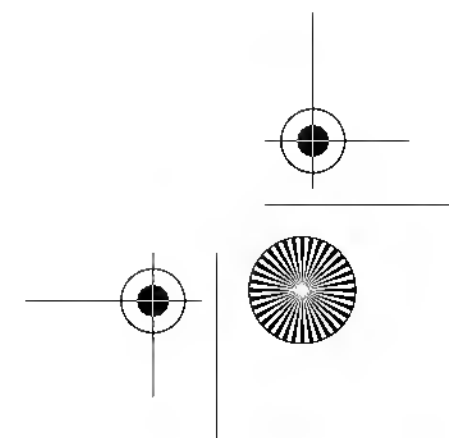
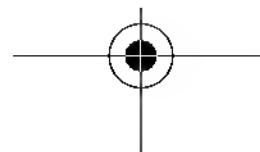
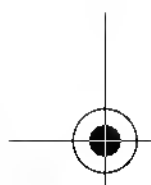
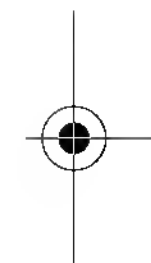
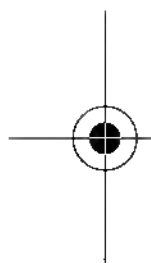


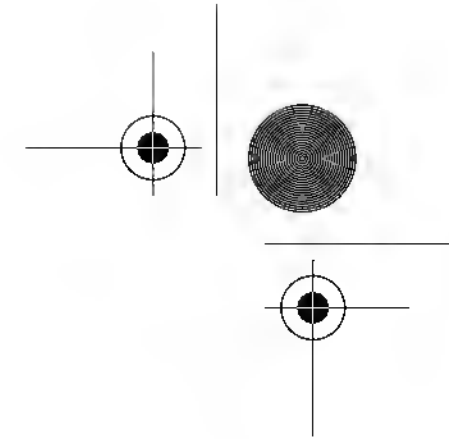
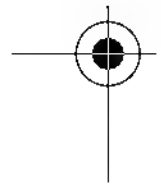
157. Ross, T. 1996. Indices for performance evaluation of predictive models in food microbiology. *J. Appl. Bacteriol.* 81:501–508.
158. Ross, T., P. Dalgaard, and S. Tienungoon. 2000. Predictive modelling of the growth and survival of *Listeria* in fishery products. *Int. J. Food Microbiol.* 62:231–245.
159. Ross, T. and J. Olley. 1997. Problems and solutions in the application of predictive microbiology. In *Seafood Safety, Processing, and Biotechnology*, Shahidi, F., Jones, Y., and Kitts, D.D. (Eds.). Technomic Publishing Company Inc., Lancaster, PA, pp. 101–118.
160. Ross, T., T.A. McMeekin, and J. Baranyi. 1999. Predictive microbiology and food safety. In *Encyclopedia of Food Microbiology*, Robinson, R.K., Batt, C.A., and Patel, P. (Eds.). Academic Press, New York, pp. 1699–1710.
161. Ross, W.H., H. Couture, A. Hughes, T. Gleeson, and R.C. McKellar. 1998. A non-linear mixed effects model for the destruction of *Enterococcus faecium* in a high-temperature short-time pasteurizer. *Food Microbiol.* 15:567–575.
162. Rosso, L. and T.P. Robinson. 2001. A cardinal model to describe the effect of water activity on the growth of moulds. *Int. J. Food Microbiol.* 63:265–273.
163. Schaffner, D.W. and T.P. Labuza. 1997. Predictive microbiology: where are we, and where are we going? *Food Tech.* 51:95–99.
164. Skandamis, P.N. and G.J.E. Nychas. 2000. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant salad at various temperatures, pHs, and oregano essential oil concentrations. *Appl. Environ. Microbiol.* 66:1646–1653.
165. Skandamis, P., E. Tsigarida, and G.J.E. Nychas. 2000. Ecophysiological attributes of *Salmonella typhimurium* in liquid culture and within a gelatin gel with or without the addition of oregano essential oil. *World J. Microbiol. Biotech.* 16(1):31–35.
166. Sofos, J.N. and F.F. Busta. 1981. Antimicrobial activity of sorbate. *J. Food Prot.* 44:614–622, 647.
167. Sorrells, K.M., D.C. Enigl, and J.R. Hatfield. 1989. Effect of pH, acidulant, time, and temperature on the growth and survival of *Listeria monocytogenes*. *J. Food Prot.* 52:571–573.
168. Stecchini, M.L., M. Del Torre, S. Donda, and E. Maltini. 2000. Growth of *Bacillus cereus* on solid media as affected by agar, sodium chloride, and potassium sorbate. *J. Food Prot.* 63:926–929.
169. Steeg, P.F.T. and H.G.A.M. Cuppers. 1995. Growth of proteolytic *Clostridium botulinum* in process cheese products. II. Predictive modeling. *J. Food Prot.* 58:1100–1108.
170. Sutherland, J.P., A. Aherne, and A.L. Beaumont. 1996. Preparation and validation of a growth model for *Bacillus cereus*: the effects of temperature, pH, sodium chloride, and carbon dioxide. *Int. J. Food Microbiol.* 30:359–372.
171. Sutherland, J.P. and A.J. Bayliss. 1994. Predictive modelling of growth of *Yersinia enterocolitica*: the effects of temperature, pH and sodium chloride. *Int. J. Food Microbiol.* 21:197–215.
172. Sutherland, J.P., A.J. Bayliss, and D.S. Braxton. 1995. Predictive modelling of growth of *Escherichia coli* O157:H7: the effects of temperature, pH and sodium chloride. *Int. J. Food Microbiol.* 25:29–49.
173. Sutherland, J.P., A.J. Bayliss, D.S. Braxton, and A.L. Beaumont. 1997. Predictive modelling of *Escherichia coli* O157:H7: inclusion of carbon dioxide as a fourth factor in a pre-existing model. *Int. J. Food Microbiol.* 37:113–120.
174. Giffel, M.C. and M.H. Zwietering. 1999. Validation of predictive models describing the growth of *Listeria monocytogenes*. *Int. J. Food Microbiol.* 46:135–149.





175. ter Steeg, P.F., F.H. Pieterman, and J.C. Hellemons. 1995. Effects of air/nitrogen, temperature and pH on energy-dependent growth and survival of *Listeria innocua* in continuous culture and water-in-oil emulsions. *Food Microbiol.* 12:471–485.
176. Thomas, L.V. and J.W.T. Wimpenny. 1993. Method for investigation of competition between bacteria as a function of three environmental factors varied simultaneously. *Appl. Environ. Microbiol.* 59:1991–1997.
177. Thomas L.V., J.W.T. Wimpenny, and G.C. Barker. 1997. Spatial interactions between subsurface bacterial colonies in a model system: a territory model describing the inhibition of *Listeria monocytogenes* by a nisin-producing lactic acid bacterium. *Microbiology (UK)* 143(Part 8): 2575–2582.
178. Thomas, L.V., J.W.T. Wimpenny, and J.G. Davis. 1993. Effect of three preservatives on the growth of *Bacillus cereus*, verocytotoxigenic *Escherichia coli* and *Staphylococcus aureus* on plates with gradients of pH and sodium chloride concentration. *Int. J. Food Microbiol.* 17:289–301.
179. Thomas, L.V., J.W.T. Wimpenny, and A.C. Peters. 1991. An investigation of four variables on the growth of *Salmonella typhimurium* using two types of gradient gel plates. *Int. J. Food Microbiol.* 14:261–275.
180. Thomas, L.V., J.W.T. Wimpenny, and A.C. Peters. 1992. Testing multiple variables on the growth of a mixed inoculum of *Salmonella* strains using gradient plates. *Int. J. Food Microbiol.* 15:165–175.
181. Tienungoon, S., D.A. Ratkowsky, T.A. McMeekin, and T. Ross. 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Appl. Environ. Microbiol.* 66:4979–4987.
182. Troller, J.A. 1983. Effect of low moisture environments on the microbial stability of foods. In *Food Microbiology*, Rose, A.H. (Ed.). Academic Press, London, pp. 173–198.
183. Tsai, Y.W. and S.C. Ingham. 1997. Survival of *Escherichia coli* O157:H7 and *Salmonella* spp. in acidic condiments. *J. Food Prot.* 60:751–755.
184. Tuynenburg Muys, G. 1971. Microbial safety in emulsions. *Process Biochem.* 6(6):25–28.
185. Valik, L. and E. Pieckova. 2001. Growth modelling of heat-resistant fungi: the effect of water activity. *Int. J. Food Microbiol.* 63:11–17.
186. Verrips, C.T. and J. Zaalberg. 1980. The intrinsic microbial stability of water-in-oil emulsions. I. Theory. *Eur. J. Appl. Microbiol. Biotech.* 10:187–196.
187. Verrips, C.T., D. Smid, and A. Kerkhof. 1980. The intrinsic microbial stability of water-in-oil emulsions. II. Experimental. *Eur. J. Appl. Microbiol. Biotech.* 10:73–85.
188. Walls, I. and V.N. Scott. 1996. Validation of predictive mathematical models describing the growth of *Escherichia coli* O157:H7 in raw ground beef. *J. Food Prot.* 59:1331–1335.
189. Walls, I. and V.N. Scott. 1997. Validation of predictive mathematical models describing the growth of *Listeria monocytogenes*. *J. Food Prot.* 60:1142–1145.
190. Walls, I., V.N. Scott, and D.T. Bernard. 1996. Validation of predictive mathematical models describing growth of *Staphylococcus aureus*. *J. Food Prot.* 59:11–15.
191. Walker, S.J., P. Archer, and J.G. Banks. 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. Appl. Bacteriol.* 68:157–162.
192. Walker, S.L., T.F. Brocklehurst, and J.W.T. Wimpenny. 1997. The effects of growth dynamics upon pH gradient formation within and around subsurface colonies of *Salmonella typhimurium*. *J. Appl. Microbiol.* 82:610–614.
193. Walker, S.L., T.F. Brocklehurst, and J.W.T. Wimpenny. 1998. Adenylates and adenylate-energy charge in submerged and planktonic cultures of *Salmonella enteritidis* and *Salmonella typhimurium*. *Int. J. Food Microbiol.* 44:107–113.





194. Whiting, R.C. 1997. Microbial database building: what have we learned? *Food Tech.* 51:82–109.
195. Whiting, R.C. and M.O. Masana. 1994. *Listeria monocytogenes* survival model validated in simulated uncooked-fermented meat products for effects of nitrite and pH. *J. Food Sci.* 59:760–762.
196. Wijtzes, T., F.M. Rombouts, M.L.T. Kant-Muermans, K. Van't Riet, and M.H. Zwietering. 2001. Development and validation of a combined temperature, water activity, pH model for bacterial growth rate of *Lactobacillus curvatus*. *Int. J. Food Microbiol.* 63:57–64.
197. Wilson, P.D.G., T.F. Brocklehurst, S. Arino, D. Thuault, M. Jakobsen, M. Lange, J. Farkas, J.W.T. Wimpenny, and J.F. Van Impe. 2002. Modelling microbial growth in structured foods: towards a unified approach. *Int. J. Food Microbiol.* 73:275–289.
198. Wilson, P.D.G., D.R. Wilson, and C.R. Waspe. 2000. Weak acids: disassociation in complex buffering systems and partitioning into oils. *J. Sci. Food Agric.* 80:471–476.
199. Wimpenny, J.W.T. 1979. The growth and form of bacterial colonies. *J. Gen. Microbiol.* 114:483–486.
200. Wimpenny, J.W.T. and J.P. Coombs. 1983. Penetration of oxygen into bacterial colonies. *J. Gen. Microbiol.* 129:1239–1242.
201. Wimpenny, J.W.T., L. Leistner, L.V. Thomas, A.J. Mitchell, K. Katsaras, and P. Peetz. 1995. Submerged bacterial colonies within food and model systems: their growth, distribution and interactions. *Int. J. Food Microbiol.* 28:299–315.
202. Wimpenny, J.W.T. and M.W.A. Lewis. 1977. The growth and respiration of bacterial colonies. *J. Gen. Microbiol.* 103:9–18.
203. Wimpenny, J.W.T. and P. Waters. 1984. Growth of microorganisms in gel-stabilized two-dimensional gradient systems. *J. Gen. Microbiol.* 130:2921–2926.
204. Wimpenny, J.W.T. and P. Waters. 1987. The use of gel-stabilized gradient plates to map the responses of microorganisms to three or four factors varied simultaneously. *FEMS Microbiol. Lett.* 40:263–267.
205. Windholz, M. 1983. *The Merck Index*. Merck and Co Inc., Rahway, NJ.
206. Yu, L.S. and D.Y.C. Fung. 1993. Five-tube most-probable-number method using the Fung-Yu tube for enumeration of *Listeria monocytogenes* in restructured meat products during refrigerated storage. *Int. J. Food Microbiol.* 18(2):97–106.
207. Zaika, L.L., J.G. Phillips, J.S. Fanelli, and O.J. Scullen. 1998. Revised model for aerobic growth of *Shigella flexneri* to extend the validity of predictions at temperatures between 10 and 19°C. *Int. J. Food Microbiol.* 41:9–19.
208. Zaroni, B., C. Peri, C. Garzaroli, and S. Pierucci. 1997. A dynamic mathematical model of the thermal inactivation of *Enterococcus faecium* during bologna sausage cooking. *Food Sci. Technol. Lebensm. Wiss.* 30:727–734.
209. Zhao, L., T.J. Montville, and D.W. Schaffner. 2000. Inoculum size of *Clostridium botulinum* 56A spores influences time-to-detection and percent growth-positive samples. *J. Food Sci.* 65:1369–1375.
210. Zhao, L., T.J. Montville, and D.W. Schaffner. 2002. Time to detection, percent-growth-positive and maximum growth rate models for *Clostridium botulinum* 56A at multiple temperatures. *Int. J. Food Microbiol.* 77:187–197.

