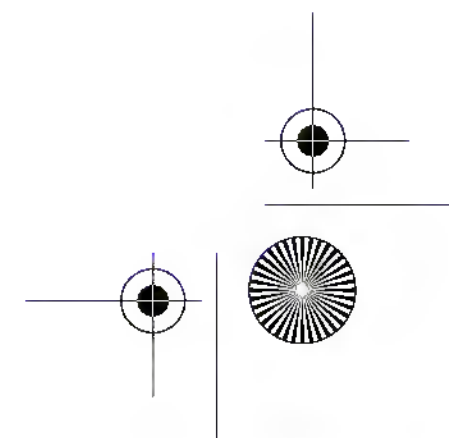
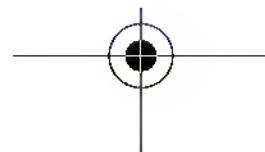
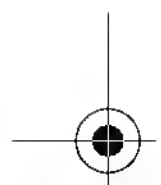
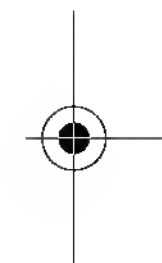
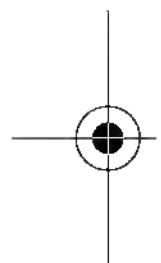


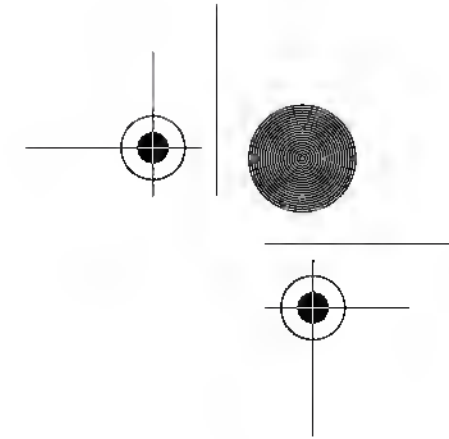
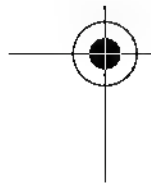
3 Secondary Models

Thomas Ross and Paw Dalgaard

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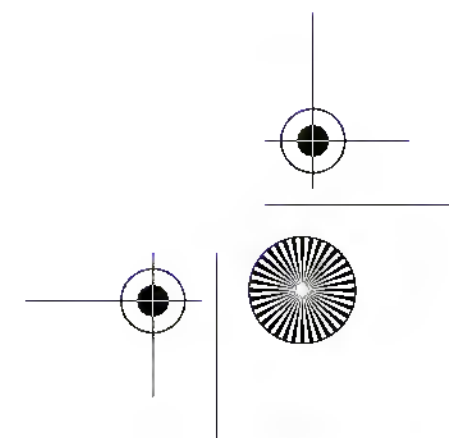
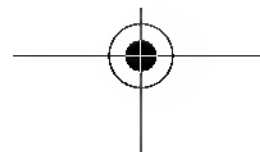
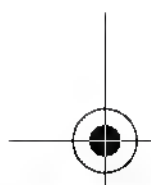
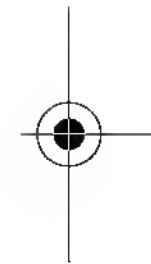
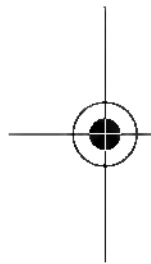
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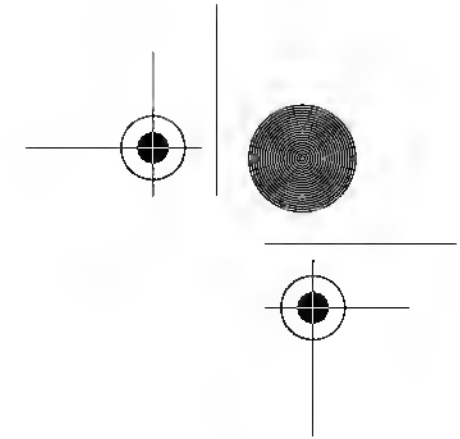
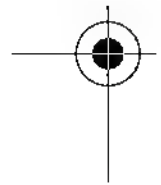
3.1 INTRODUCTION

Changes in populations of microorganisms in foods over time (i.e., “microbial kinetics”) are governed by storage conditions (“extrinsic” factors) and product characteristics (“intrinsic” factors). Collectively these have been termed “environmental parameters.” They may represent simple situations, e.g., where the storage temperature is the only important factor influencing microbial kinetics, but in many foods the environmental parameters that influence microbial kinetics are complex and dynamic and include the combined effects of extrinsic factors such as temperature and storage atmosphere; intrinsic factors such as water activity, pH, naturally occurring organic acids, and added preservatives; and interactions between groups of microorganisms.

Consistent with the widely accepted terminology introduced by Whiting and Buchanan (1993), we term those models that describe the response of microorganisms to a single set of conditions *over time* as “primary” models (see [Chapter 2](#)). Models that describe the effect of environmental conditions, e.g., physical, chemical, and biotic features, on the values of the parameters of a primary model are termed “secondary” models.

Knowledge of the environmental parameters that most influence growth of microorganisms in foods is essential for the development, as well as for the practical use, of predictive microbiology models. Secondary models that do not include all the environmental parameters important in a food are said to be “incomplete” (Ross, Baranyi, McMeekin, 2000) and require expansion (or simple “calibration” if those factors are constant) to accommodate their effect on microbial kinetics. The environmental parameters that are important for particular foods, however, are not always known. In those situations the systematic approach of predictive microbiology can help to elucidate the microbial ecology of the product.





In this chapter we consider a range of types of secondary models including those that model the probability that a predicted kinetic response will occur. The chapter includes descriptions and comparison of models, as considerations for development of robust, secondary models. [Appendix A3.1](#) details methods to measure environmental factors of importance — an essential element of the application of predictive microbiology.

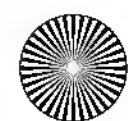
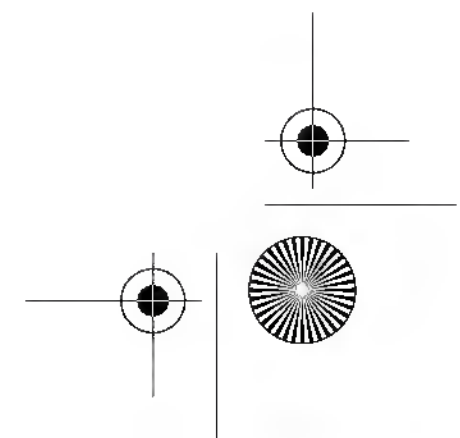
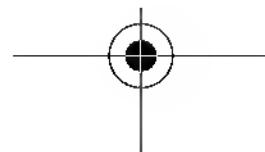
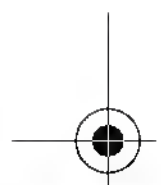
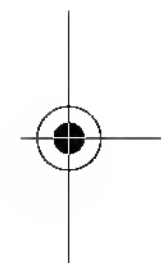
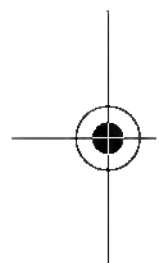
3.1.1 PHILOSOPHY, TERMINOLOGY, AND METHODOLOGY

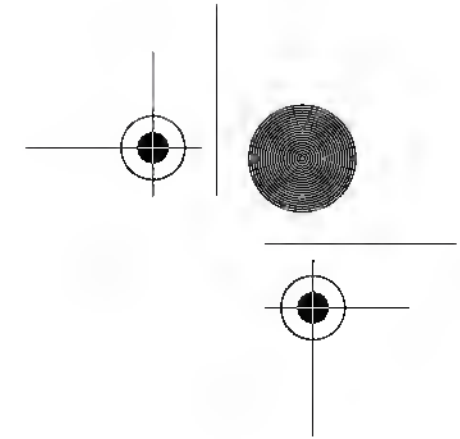
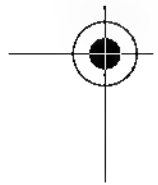
The history of predictive microbiology, including the philosophical motivations of Roberts and Jarvis (1983), who first proposed the concept, was traced by Ross and McMeekin (1994). From a purely pragmatic perspective, predictive microbiology aims to collect and make accessible computerized data on the behavior of microbial populations in response to defined environmental conditions, but mathematical modeling also provides a useful and rigorous framework for the hypothetico-deductive scientific process. To develop a consistent framework that enables us to understand and predict the microbial ecology of foods it is desirable to integrate the patterns of microbial behavior revealed in predictive modeling studies with knowledge of the physiology of microorganisms and physical and chemical processes and phenomena that occur in foods and food processes (Ross, Baranyi, McMeekin, 2000).

Various types and categorizations of models are recognized. Empirical models are, essentially, pragmatic and simply describe a set of data in a convenient mathematical relationship with no consideration of underlying phenomena. Mechanistic models are built up from theoretical bases and, if they are correctly formulated, can allow the response to be interpreted in terms of known physical, chemical, and biological phenomena. An advantage of mechanistic approaches is that they tend to provide a better foundation for subsequent development and expansion of models; i.e., taken to their logical extreme, models for specific situations would simply be special, or reduced, cases of a much larger and holistic model that describes, quantitatively, the microbial ecology of foods. The process of developing models that are able to be integrated readily with other models so as to describe more complex phenomena has been termed “nesting” or “embedding.” A fuller explanation of the benefits of that approach was provided by Baranyi and Roberts (1995).

In one sense, a model is the mathematical expression of a hypothesis. If this approach is adopted, it follows that the parameters in such models might be readily interpretable properties of the system under study, and that the mathematical form of the model would enable interpretation of the interactions between those factors. Interpretability of model parameters is a feature highly valued by many authors in the predictive microbiology literature (e.g., Augustin and Carlier, 2000a,b; Rosso et al., 1993; Wijtzes et al., 1995). Although the development of predictive microbiology has seen the embedding of more and more mechanistic elements, or at least models whose structure and parameterization reflects known or hypothesized underlying phenomena, in practice many models currently available in predictive microbiology are not purely empirical, and none are purely mechanistic (Ross, Baranyi, McMeekin, 2000).

Another, often cited, advantage of mechanistic models is that if they are built on sound theory they are more likely to facilitate prediction by extrapolation. Conversely,





as none of the models in use in predictive microbiology can be considered to be mechanistic, they can only be used to make predictions by interpolation. (Determination of the interpolation region encompassed by a model is discussed in Sections 3.2.5 and 3.4.3.4.) It is perhaps ironic, then, that 20 years of experience in predictive microbiology has not demonstrated the practical usefulness of mechanistic models that have been proposed to date (see Section 3.2.4). In general, even with good quality data the mechanistic models do not provide better fit and are usually harder to work with than quasi-mechanistic or empirical models currently used.

Predictive microbiology is a specific application of the field of mathematical modeling and, as such, the same rules of modeling as are applied in those other disciplines are relevant to the development of predictive food microbiology models. These have been discussed by various authors (Draper and Smith, 1981; McMeekin et al., 1993; Ratkowsky, 1993), and an overview is presented in [Table 3.1](#).

Experimental methods and design considerations relevant to kinetic models were discussed in detail in McMeekin et al. (1993; [Chapter 2](#)), Davies (1993), and Legan et al. (2002) and are also discussed in [Chapter 1](#). Two points that we feel are necessary to reiterate are the limitations of the central composite design in predictive microbiology studies, and consideration of spoilage domains when growth of spoilage microorganisms is studied. Legan et al. (2002) accentuated the importance of experimental design in growth modeling studies stating:

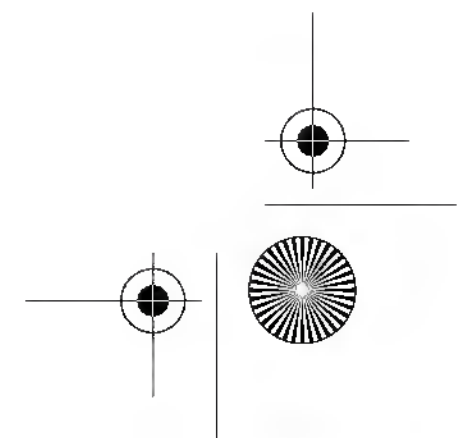
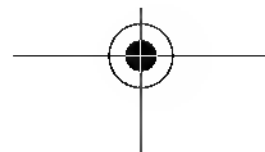
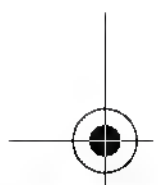
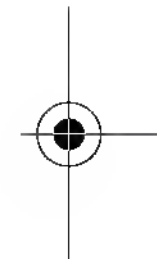
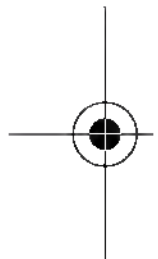
in other disciplines, such as engineering, central composite designs are commonly used for developing response surface models. For microbiological modeling, however, these designs have serious limitations and should be avoided. Central composite designs concentrate treatments in the centre of the design space and have fewer treatments in the extreme regions where biological systems tend to exhibit much greater variability.

Microbial food spoilage is dynamic and in some cases relatively small changes in environmental parameters cause a complete shift in the micro or a responsible for product spoilage. Thus, to avoid modeling growth of spoilage microorganisms under conditions where they have no influence on quality, a product-oriented approach that includes determination of the spoilage domain of specific microorganisms is often required (Dalgaard, 2002).

We will not comment further on methodology appropriate to development of kinetic models, other than to say that to develop reliable secondary models an understanding of microbial physiology and its interaction with food environments and storage and processing conditions must be borne in mind in the design of experiments including the preparation of cultures and interpretation of measurements of population changes. This issue is particularly explored and exemplified in Section 3.4.4.4 concerning experimental considerations relevant to the development of growth limits models.

3.2 SECONDARY MODELS FOR GROWTH RATE AND LAG TIME

Implicit in the appropriate development and use of secondary models in predictive food microbiology is the ability to characterize foods, and the environment they



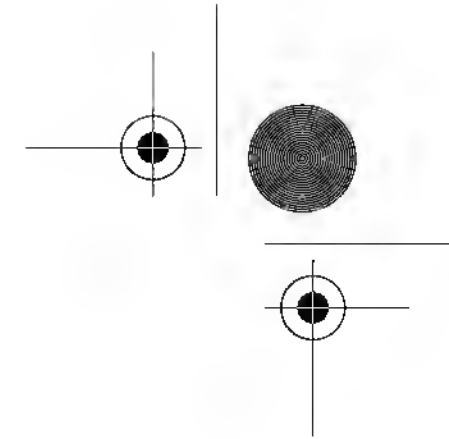
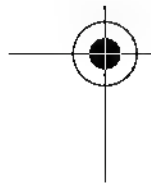
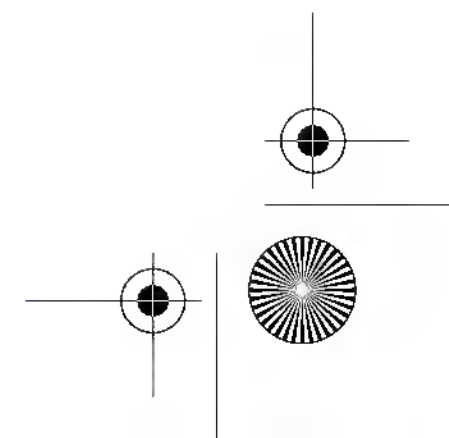
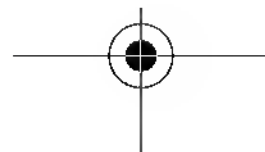
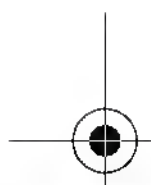


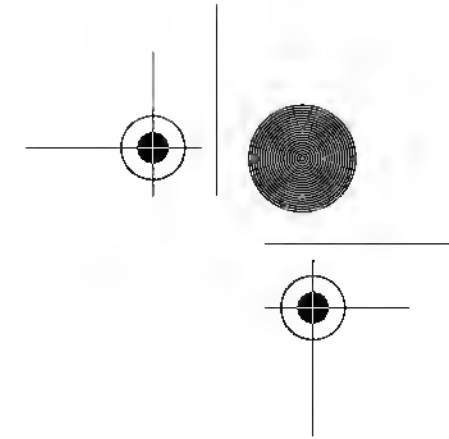
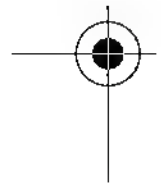
TABLE 3.1
Some Considerations in the Selection of Models

| Subject | Reasons |
|--------------------------------------|---|
| Parameter estimation properties | Relates to the procedure and reliability of estimating the model parameters. In general, models should have parameters that are independent, identically distributed, normal or “iidn” (see, e.g., Ratkowsky, 1993) |
| Stochastic assumption | The form of the model, and choice of response variables, should be such that the difference between prediction and observations (or some mathematical transformation of them) is normally distributed, and that the magnitude of the error is independent of the magnitude of the response modeled. If not, the fitting can be dominated by some data, at the expense of other data |
| Parameter interpretability | As noted in the text, it is useful if the parameters have biological/physical/chemical interpretations that can be readily related to the independent and dependent variables. This can simplify the process of model creation and also aid in understanding of the model (This may be less important than the behavior and performance of the model.) |
| Parsimony | Models should have no more parameters than are required to describe the underlying behavior studied. Too many parameters can lead to a model that fits the error in the data, i.e., generates a model that is specific to a particular set of observations. Nonparsimonious models have poor predictive ability |
| Interpolation region | No models in predictive microbiology can be considered to be mechanistic and predictions can be made by interpolation only. Thus, the interpolation region defines the useful range of applicability of the model. The interpolation region is affected by not only the range of individual variables, but also the experimental design (see Section 3.2.5) |
| Correct qualitative features | In mathematical terms, these are the analytical properties of the model function. They include convexity, monotonicity, locations of extreme, and zero values. If biological considerations prescribe any of these, the model should reflect those properties accurately |
| “Extendibility” (embedding, nesting) | When a model is developed further (such as to include more or dynamically changing environmental factors) the new, more complex model should embody the old, simpler model as a special case |

Source: Modified from Ross, T., Baranyi, J., and McMeekin, T.A. In *Encyclopaedia of Food Microbiology*, Robinson, R., Batt, C.A., and Patel, P. (Eds.), Academic Press, London, 2000, pp. 1699–1710.

present to contaminating microorganisms, in terms of those biotic and abiotic elements that affect the dynamics of the microbial population of interest. Methods to characterize the physicochemical environment, including temperature, gaseous atmosphere, salt and/or water activity, pH and organic acids, spices, smoke, and other components, are discussed in detail in [Appendix A3.1](#). These topics are also considered in [Chapter 5](#), including discussion of the influence of other organisms and heterogeneity in the environment. Another important element is the ability to characterize temporal changes in the environment. Techniques for modeling microbial population dynamics under time-varying conditions are considered in [Chapter 7](#).





Within predictive microbiology the development and application of secondary models for growth rates and lag times have been extensively reviewed (Buchanan, 1993b; Davey, 1999; Farber, 1986; ICMSF, 1996a,b; McDonald and Sun, 1999; McMeekin et al., 1993; Ross, 1999a,b; Ross and McMeekin, 1994; Skinner et al., 1994; Whiting, 1995). This section describes types of secondary growth rate and lag time models that are currently available, but with particular focus on more recent developments, and also includes a detailed tabulation of models available for specific microorganisms.

3.2.1 SQUARE-ROOT-TYPE MODELS

3.2.1.1 Temperature

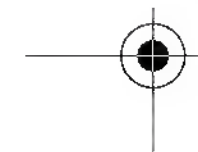
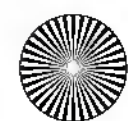
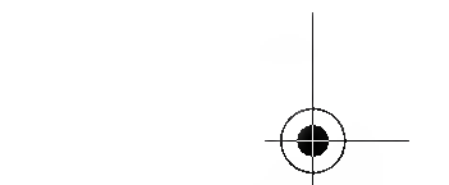
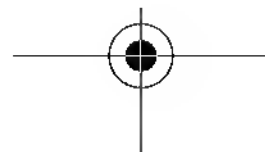
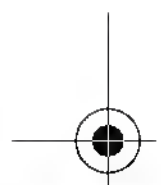
As discussed later (Section 3.2.4), in many cases the classical Arrhenius equation is inappropriate to describe the effect of suboptimal temperature on growth rates of microorganisms because the (apparent) activation energy (E_a) itself is temperature dependent. To overcome this problem Ratkowsky et al. (1982) suggested a simple empirical model (Equation 3.1). When this model was fitted to experimental growth rates the data were square-root transformed to stabilize their variance and this simple model and its numerous expansions are named square-root-type, Ratkowsky-type, or Bêlerádek-type models (McMeekin et al., 1993). These models, and the closely related cardinal parameter models (see Section 3.2.3), are probably the most important group of the secondary models within predictive microbiology.

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \quad (3.1)$$

where b is a constant and T is the temperature. The parameter T_{\min} , a theoretical minimum temperature for growth, is the intercept between the model and the temperature axis (Figure 3.1). T_{\min} is a model parameter and its value can be 5 to 10°C lower than the lowest temperature at which growth is actually observed. This interpretation differs from that embodied in the cardinal parameter models, as discussed in Section 3.2.3 and Chapter 4).

From growth rates measured at several different constant temperatures the values of b and T_{\min} in Equation 3.1 can be determined by classical model fitting techniques (see Chapter 4). Recently it was suggested that b and T_{\min} could be estimated from a single, optimally designed, experiment where growth resulting from a temperature profile is recorded (Bernaerts et al., 2000). These authors concluded that such an optimal, dynamic, one-step experiment would reduce the experimental work required to develop a model significantly and would have substantial potential within predictive microbiology. So far this technique has not found wider use within predictive microbiology and its ability to estimate model parameters accurately remains to be confirmed for different microorganisms and environmental parameters.

Ratkowsky et al. (1983) expanded Equation 3.1 to include the entire biokinetic range of growth temperatures (Equation 3.2, Figure 3.1). From this model the optimal



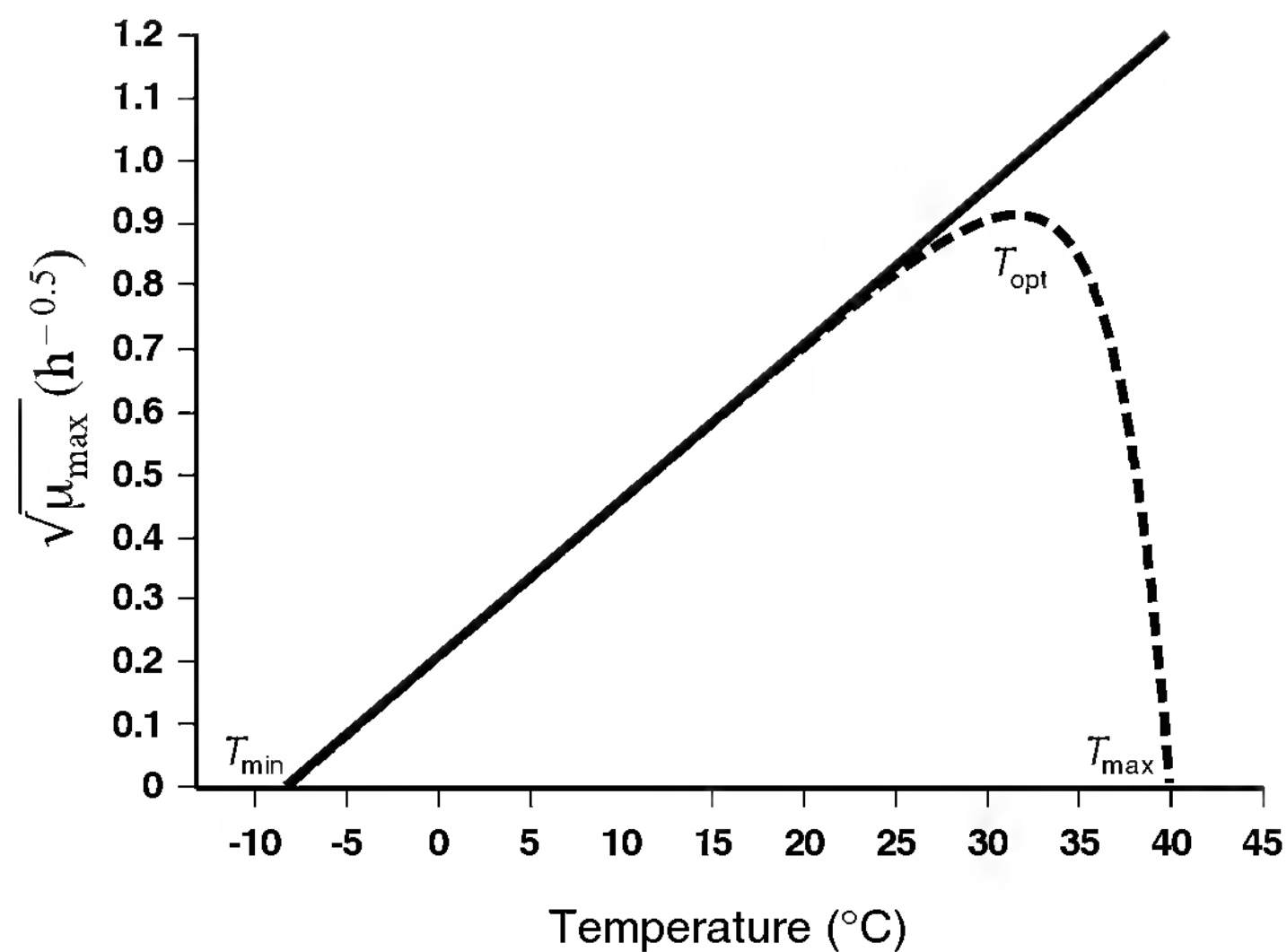
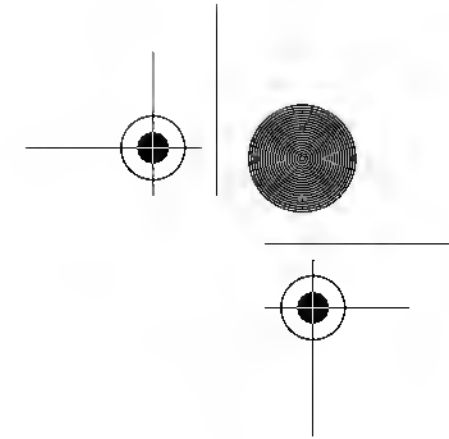
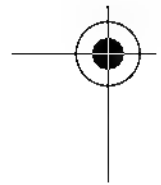


FIGURE 3.1 Simulation of Equation 3.1 (solid line) and Equation 3.2 (dashed line). $b = 0.025 \text{ h}^{0.5}/^\circ\text{C}$, $T_{\min} = -8^\circ\text{C}$, $c = 0.30^\circ\text{C}^{-1}$, and $T_{\max} = 40^\circ\text{C}$.

growth temperature can be determined by solving the following equation: $c \times (T_{\text{opt}} - T_{\min}) = \exp[c \times (T_{\text{opt}} - T_{\min})] - 1$ (McMeekin et al., 1993).

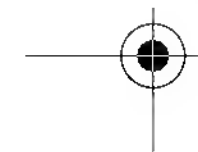
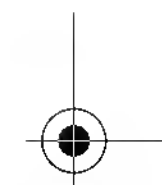
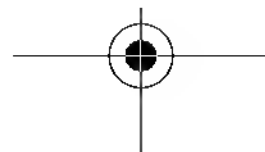
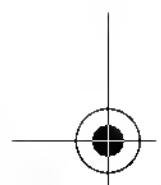
$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \cdot (1 - \exp(c(T - T_{\max}))) \quad (3.2)$$

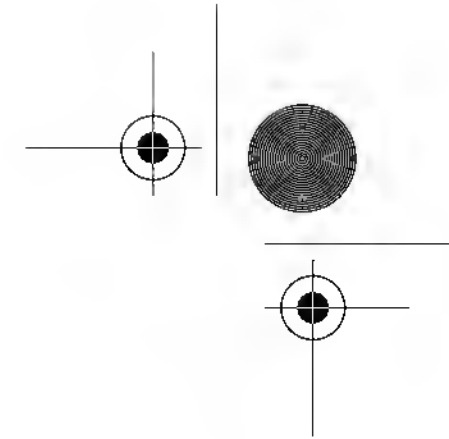
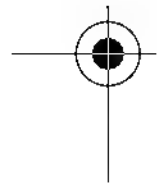
where b and c are constants, T is the temperature, T_{\min} the theoretical minimum temperature below which no growth is possible, and T_{\max} is the theoretical maximum temperature beyond which growth is not possible.

While Ratkowsky et al. (1982, 1983) settled for an exponent of 2, the original Bêlerádek models had a variable exponent value. Dantigny (1998) and Dantigny and Molin (2000) used the concepts of dimensionless growth rate variables (effectively the same as the gamma factor concept; see Section 3.2.3) to explore the most appropriate value of the exponent for bacterial growth rate data using Bêlerádek-type models. They reported a correlation between the estimate of T_{\min} and the exponent value used and found that when T_{\min} and the exponent were simultaneously fitted by nonlinear regression, thermophiles had lower fitted exponent values than did mesophiles or psychrotrophic organisms. They reported that the use of the square-root model leads to an underestimation of the minimum temperature for growth when the exponent value is significantly less than 2.

3.2.1.2 Water Activity

McMeekin et al. (1987) found that growth responses of *Staphylococcus xylosus* followed Equation 3.1 at different values of water activity. T_{\min} was constant and





thus independent of water activity and Equation 3.3 was suggested to describe the combined effect of temperature and water activity (McMeekin et al., 1987).

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \cdot \sqrt{a_w - a_{w \min}} \quad (3.3)$$

where b and T_{\min} are as previously defined, a_w is the water activity, and $a_{w \min}$ is the theoretical minimum water activity below which growth is not possible.

Later, Miles et al. (1997) suggested that Equation 3.4 be used to take into account the effect of the entire biokinetic ranges of both temperature and water activity.

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \cdot (1 - \exp(c(T - T_{\max}))) \cdot \sqrt{(a_w - a_{w \min})(1 - \exp(d(a_w - a_{w \max})))} \quad (3.4)$$

where b , c , T , T_{\min} , T_{\max} , a_w , and $a_{w \min}$ are as previously defined, d is a fitted constant, and $a_{w \max}$ is a theoretical maximum water activity beyond which growth is not possible.

Most food-related microorganisms grow at water activities very close to 1.000 and in those cases the expanded water activity term (i.e., containing $a_{w \max}$) in Equation 3.4 is not needed to predict growth in foods. However, some microorganisms, e.g., several marine bacteria, have a substantial requirement for minerals. To model growth responses of these microorganisms, the inhibitory effect of high water activities, i.e., low salt concentrations, must be taken into account. For the human pathogen *Vibrio parahaemolyticus*, $a_{w \max}$ has been determined to be 0.998. Some seafood spoilage bacteria are more inhibited by high water activity; e.g., growth of *Halobacterium salinarium* was only observed at a_w values below 0.9 (Chandler and McMeekin, 1989; Doe and Heruwati, 1988; Miles et al., 1997).

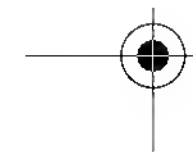
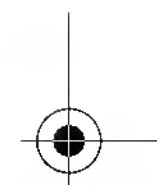
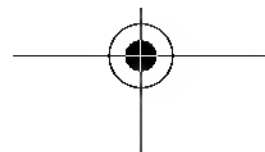
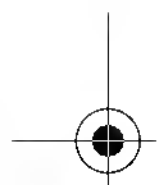
3.2.1.3 pH

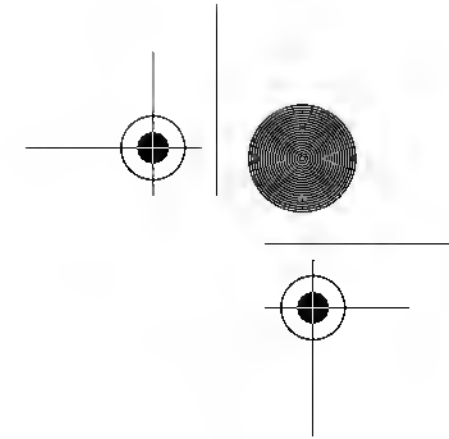
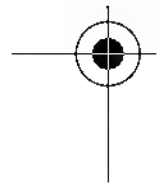
For *Yersinia enterocolitica*, Adams et al. (1991) found that growth responses followed Equation 3.1 at different values of pH. Again, T_{\min} was constant and Equation 3.5 was suggested.

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \cdot \sqrt{pH - pH_{\min}} \quad (3.5)$$

where pH_{\min} is the theoretical minimum pH below which growth is not possible and other parameters are as previously defined.

On the basis of the observation of Cole et al. (1990) that growth rate was proportional to hydrogen ion concentration, Presser et al. (1997) introduced the following quasi-mechanistic term to describe the effect of pH on bacterial growth:





$$\mu_{\max} = \mu_{opt} \times (1 - 10^{pH_{\min} - pH}) \quad (3.6a)$$

By analogy, another term was introduced for superoptimal (i.e., alkaline) pH conditions, leading to the following model for the entire biokinetic pH range:

$$\mu_{\max} = \mu_{opt} \times (1 - 10^{pH_{\min} - pH}) \times (1 - 10^{pH - pH_{\max}}) \quad (3.6b)$$

The validity of that term was evaluated against an extensive data set for *Escherichia coli* growth, including variables of temperature, water activity, and lactic acid concentration for a range of acid and alkaline environmental pH levels (see Equation 3.10).

Wijtzes et al. (1995, 2001) continued the development of square-root-type models and suggested Equation 3.7 for growth responses of *Lactobacillus curvatus* at different temperatures, a_w values, and pH

$$\mu = b \cdot (a_w - a_{w \min}) \cdot (pH - pH_{\min}) \cdot (pH - pH_{\max}) \cdot (T - T_{\min})^2 \quad (3.7)$$

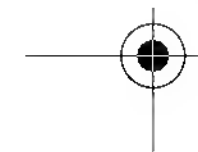
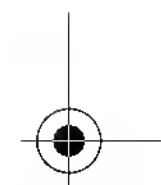
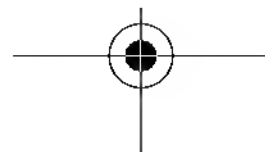
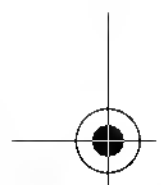
3.2.1.4 Other Factors

Equation 3.8 was suggested to model the effect of carbon dioxide-enriched (%CO₂) atmospheres on growth of the specific spoilage organism *Photobacterium phosphoreum* on fish (Dalgaard, 1995; Dalgaard et al., 1997). Later, similar but square-root-transformed terms were used to model the effect of CO₂ and sodium lactate (NaL) on growth of *Lactobacillus sake* and *Listeria monocytogenes* at a constant pH (Equation 3.9; Devlieghere et al., 1998, 2000a,b, 2001).

$$\sqrt{\mu_{\max}} = b(T - T_{\min}) \times \frac{(\%CO_{2 \max} - \%CO_2)}{\%CO_{2 \max}} \quad (3.8)$$

$$\begin{aligned} \sqrt{\mu_{\max}} = & b \\ & \cdot (T - T_{\min}) \\ & \cdot \sqrt{a_w - a_{w \min}} \\ & \cdot \sqrt{CO_{2 \max} - CO_2} \\ & \cdot \sqrt{NaL_{\max} - NaL} \end{aligned} \quad (3.9)$$

As noted above, a more comprehensive square-root-type model that includes the effects of temperature, pH, water activity, and lactic acid has been suggested and developed in a series of publications (Presser et al., 1997; Ross, 1993a,b; Salter et al., 1998; Tienungoon, 1988) and has been applied to *Listeria monocytogenes* and *Escherichia coli* growth rates. It was presented in its most complete form in Ross et al. (2003):



$$\begin{aligned}
\sqrt{\mu_{\max}} &= c \\
&\times (T - T_{\min}) \times (1 - \exp(d \times (T - T_{\max}))) \\
&\times \sqrt{a_w - a_{w\min}} \times (1 - \exp(g \times (a_w - a_{w\max}))) \\
&\times \sqrt{1 - 10^{pH_{\min} - pH}} \\
&\times \sqrt{1 - 10^{pH - pH_{\max}}} \\
&\times \sqrt{1 - \frac{LAC}{U_{\min} \times (1 + 10^{pH - pK_a})}} \\
&\times \sqrt{1 - \frac{LAC}{D_{\min} \times (1 + 10^{pK_a - pH})}}
\end{aligned} \tag{3.10}$$

where c , d , and g are fitted parameters, LAC is the lactic acid concentration (mM), U_{\min} the minimum concentration (mM) of undissociated lactic acid that prevents growth when all other factors are optimal, D_{\min} the minimum concentration (mM) of dissociated lactic acid that prevents growth when all other factors are optimal, pK_a is the pH for which concentrations of undissociated and dissociated lactic acid are equal, reported to be 3.86 (Budavari, 1989), and all other terms are as previously defined.

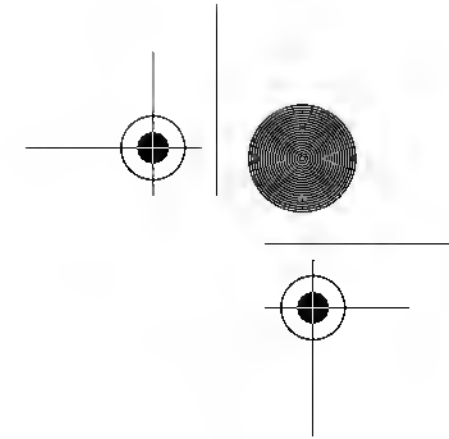
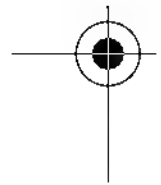
One of the advantages of the square-root-type models, and the cardinal parameters models, is that their form enables them to be readily simplified into models for special cases; e.g., in Equation 3.10, if one factor is held constant then the terms involving that factor simply reduce to constants.

An example is a model developed for *Listeria monocytogenes* (Ross et al., in press; WHO/FAO, in press), in which the superoptimal water activity term is not relevant, and in which a term for the effect of nitrite on *L. monocytogenes* growth rate was also included. That novel term was based on analysis of the predictions of the Pathogen Modeling Program (Buchanan, 1993a; www.arserrc.gov/mfs/pathogen.htm). The fitted model is shown in Equation 3.11.

$$\begin{aligned}
\sqrt{\mu_{\max}} &= 0.1626 \\
&\times (T - 0.60) \times (1 - \exp(0.129 \times (T - 51.0))) \\
&\times \sqrt{a_w - 0.925} \\
&\times \sqrt{1 - 10^{4.93 - pH}} \\
&\times \sqrt{1 - \frac{LAC}{4.55 \times (1 + 10^{pH - 3.86})}} \\
&\times \sqrt{\left(\left(493 - NIT \times \left(1 + \frac{(6.5 - pH)}{2} \right) \right) / 493 \right)}
\end{aligned} \tag{3.11}$$

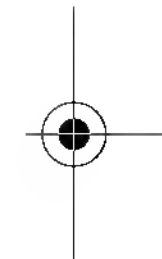
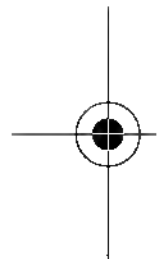
where NIT is the concentration of nitrite and all other terms are as previously defined.

As shown in Figure 3.2, Equation 3.10 and Equation 3.11 represent a new generation of square-root-type models where the level of lactic acid influences the



range of pH values for which growth is theoretically observed, reflecting the known interaction between pH and undissociated lactic acid, and also the individual growth rate suppressing effects of hydrogen ion concentration and undissociated lactic acid concentration. This was not the case for the environmental parameters included in Equation 3.1 to Equation 3.9. In those models, each term expressed how an environmental factor reduced the growth rate of a microorganism. However, for those models the expected multidimensional growth space was not influenced by levels of the different environmental parameters. This limitation of predictive models for growth rate has been recognized and has led, in part, to the development of growth/no growth models (discussed in Section 3.2.3 and Section 3.4). To make accurate predictions, a model can include terms to force the predicted growth rate to zero (Augustin and Carlier, 2000b; Le Marc et al., 2002). Alternatively, the probability of growth under the test conditions can first be assessed using a growth boundary model. If growth is possible, a growth rate model in combination with a lag time model can be used to estimate the extent of growth (Ross et al., in press; WHO/FAO, in press).

It is also notable that the pH and lactic acid terms in Equation 3.10 are effectively gamma-model type terms (see Section 3.2.2), in which the effect of the level of growth rate inhibitor is scaled between 0 and 1, where 1 represents no inhibition, i.e., the optimal level of that environmental factor. In the case of lactic acid, the optimal level would be 0, while for pH the optimum is ~7. This illustrates the close relationship between square-root-type models, and those that embody the gamma concept, such as the cardinal parameter models.



3.2.2 THE GAMMA CONCEPT

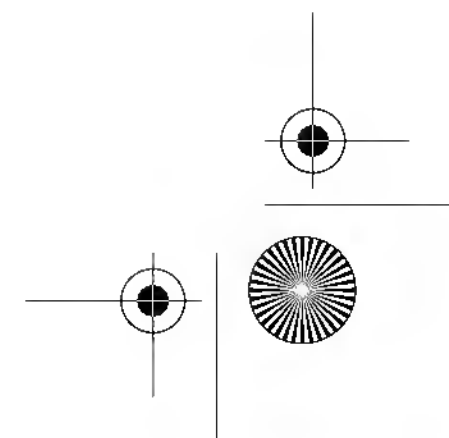
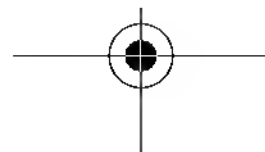
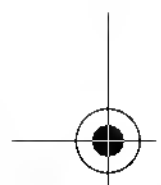
The concept of dimensionless growth factors, now known as the gamma (γ) concept, was introduced in predictive microbiology by Zwietering et al. (1992). Later, minor changes and new developments were added (Wijtzes et al., 1998, 2001; Zwietering, 1999; Zwietering et al., 1996).

The gamma (γ) concept relies on:

1. The observation (e.g., Adams et al., 1991; McMeekin et al., 1987) that many factors that affect microbial growth rate act independently, and that the effect of each measurable factor on growth rate can be represented by a discrete term that is multiplied by terms for the effect of all other growth rate affecting factors, i.e.:

$$\mu = f(\text{temperature}) \times f(a_w) \times f(\text{pH}) \times f(\text{organic acid}) \\ \times f(\text{other}_1) \times f(\text{other}_2) \times \dots \times f(\text{other}_n)$$

2. That the effect on growth rate of any factor can be expressed as a fraction of the maximum growth rate (i.e., the rate when that environmental factor is at the optimum level)



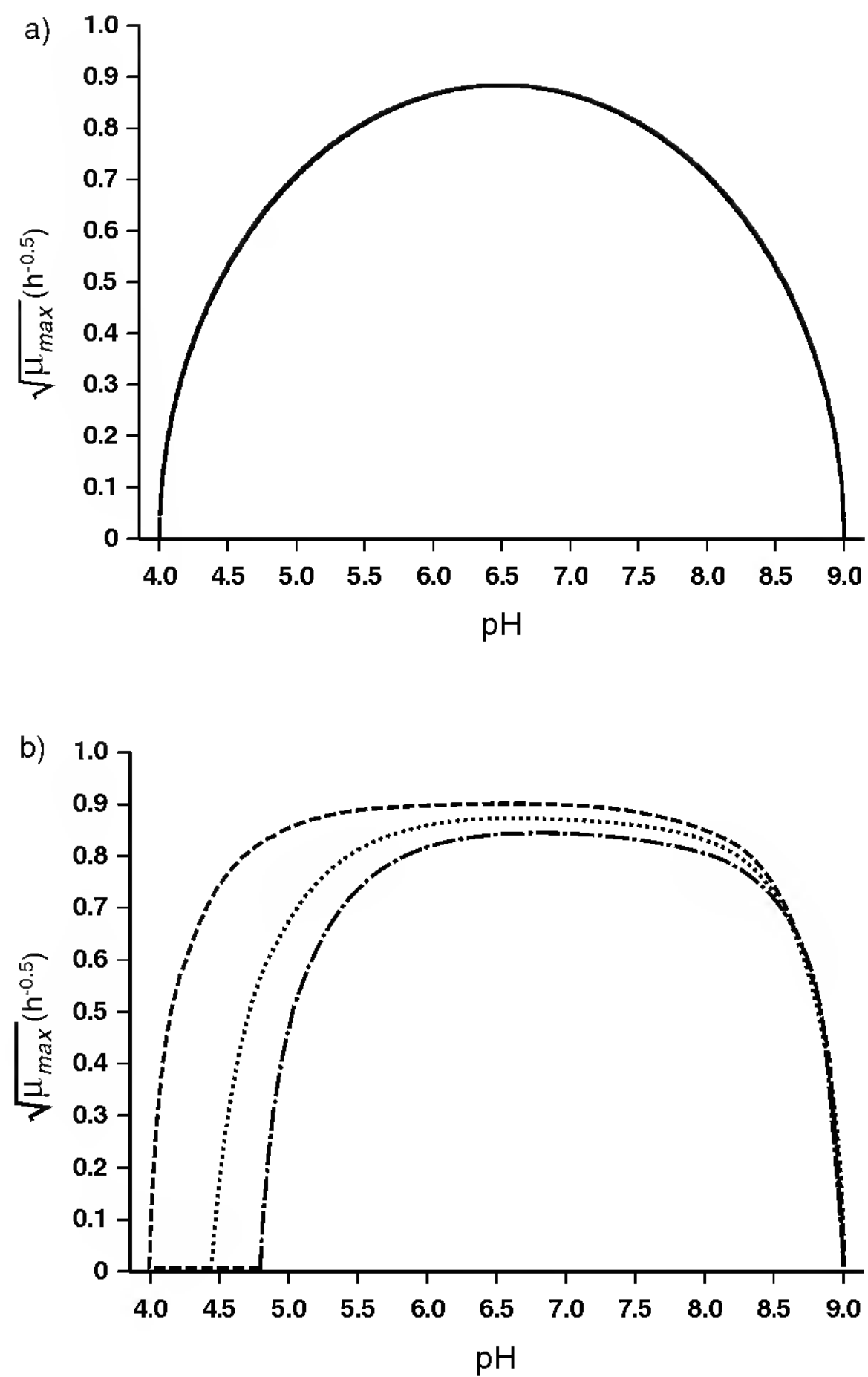
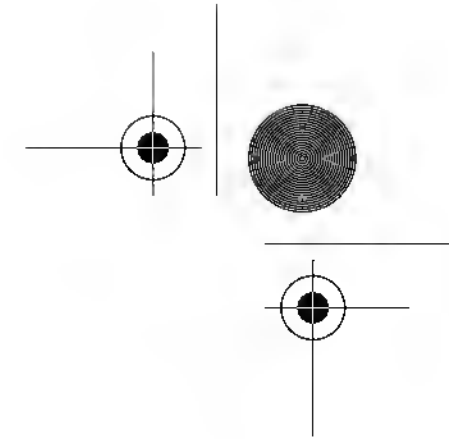
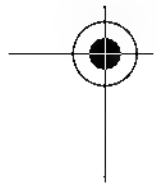
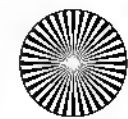
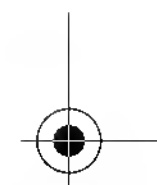
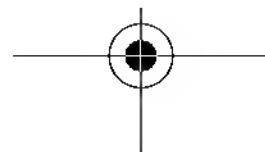
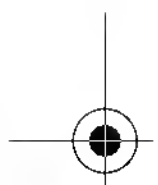
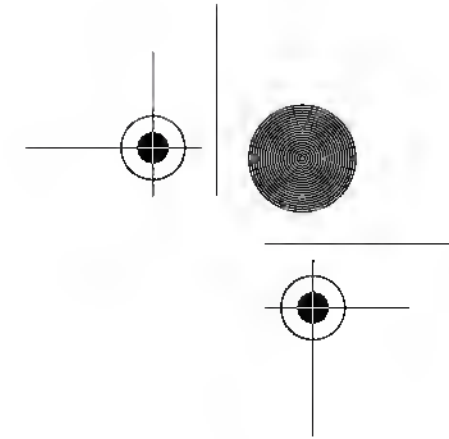
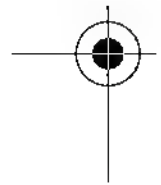


FIGURE 3.2 Simulation of Equation 3.7 (a) and Equation 3.10 (b) at a fixed temperature and water activity. pH_{min} is 4.0 and pH_{max} is 9.0. For Equation 3.10, $U_{min} = 10$ mM and $D_{max} = 1000$ mM. The concentrations of lactic acid (LAC) depicted are 0 mM (dashed line), 50 mM (dotted line), and 100 mM (dash-dotted line).

Under completely optimal conditions each microorganism has a reproducible maximum growth rate, notwithstanding the potential effect of strain variability. As any environmental factor becomes suboptimal the growth rate declines in a predictable manner, and the extent of that inhibition can be related to the optimum growth rate by calculating the relative rate at the test condition compared to that at the optimum. Thus, under the gamma concept approach, the cumulative effect of many factors poised at suboptimal levels can be estimated from the product of the relative inhibition of growth rate due to each factor, as indicated by Equation 3.12. The relative inhibitory effect of a specific environmental variable is described by a growth factor “gamma” (γ), a dimensionless measure that has a value between 0 and 1 (e.g., Equation 3.13 to Equation 3.15).





The relative inhibitory effect can be determined from the “distance” between the optimal level of the factor and the minimum (or maximum) level that completely inhibits growth by recourse to a predictive model. In the gamma model approach, the reference growth rate is μ_{\max} , so that reference levels of temperature, water activity, etc. are those that are the optimum for growth rate, usually represented as T_{opt} , $a_{w \text{ opt}}$, pH_{opt} , etc. The combined effect of several environmental factors is then determined by multiplication of their respective γ factors (Equation 3.16).

$$\begin{aligned} \gamma &= \frac{\text{Growth rate at actual environmental conditions}}{\text{Growth rate at optimal environmental conditions}} \\ &= \frac{\mu_{\max}(T, a_w, \text{pH}, \text{etc.})}{\mu_{\max \text{ opt}}} \end{aligned} \quad (3.12)$$

$$\gamma(T) = \left(\frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}} \right)^2 \quad (3.13)$$

$$\gamma(a_w) = \frac{a_w - a_{w \min}}{1 - a_{w \min}} \quad (3.14)$$

$$\gamma(\text{pH}) = \frac{(\text{pH} - \text{pH}_{\min}) \cdot ((\text{pH}_{\max} - \text{pH}))}{(\text{pH}_{\text{opt}} - \text{pH}_{\min}) \cdot (\text{pH}_{\max} - \text{pH}_{\text{opt}})} \quad (3.15)$$

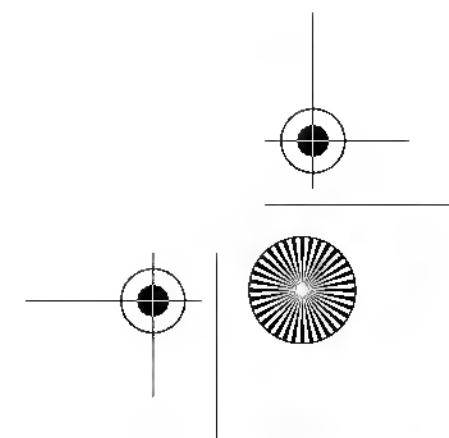
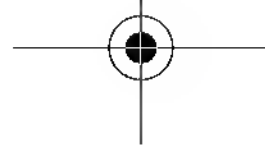
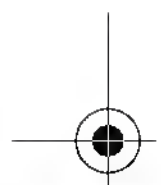
$$\mu_{\max} = \mu_{\max \text{ opt}} \cdot \gamma(T) \cdot \gamma(a_w) \cdot \gamma(\text{pH}) \quad (3.16)$$

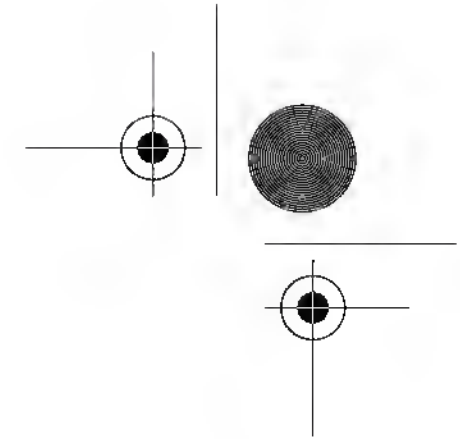
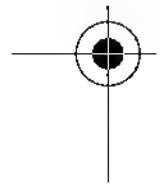
The effect of environmental parameters like carbon dioxide, sodium lactate, and nitrite has also been included in square-root-type models (see, e.g., Equation 3.8 to Equation 3.11). The absence of these inhibitory substances is optimal for growth and therefore the calculation of γ factors requires information only about the lowest concentration of each substance that prevents growth (or, similarly, the maximum level that can be tolerated before growth ceases) analogous to minimum inhibitory concentrations (MICs).

$$\gamma(\text{CO}_2) = \left(\frac{\% \text{CO}_2_{\max} - \% \text{CO}_2}{\% \text{CO}_2_{\max} - \% \text{CO}_2_{\text{opt}}} \right)^2 = \left(\frac{\% \text{CO}_2_{\max} - \% \text{CO}_2}{\% \text{CO}_2_{\max}} \right)^2 \quad (3.17)$$

3.2.2.1 Expanding Existing Models

Given that there is a finite number of models (see [Table 3.5](#) and [Table 3.6](#)), and that few models include factors of relevance to all foods, some workers have attempted to integrate terms for specific variables from one model into another to suit a specific





food and the conditions of interest. Because of the assumption of independent action of growth rate inhibitors, the dimensionless γ factors can, in principle, be readily exchanged between existing models and, at the time of writing, this is increasingly being done. Values of parameters like μ_{\max} , μ_{opt} , T_{\min} , T_{opt} , $a_{w \min}$, pH_{\min} , pH_{opt} , pH_{\max} , and $\% \text{CO}_2_{\max}$ from which gamma factors can be derived are known for a considerable number of food-related pathogenic microorganisms. The approach was possibly taken to its logical conclusion by Augustin and Carlier (2000a,b) who collated, and integrated into a single model, literature data and observations for more than 15 factors in foods that affect the growth rate of *L. monocytogenes*.

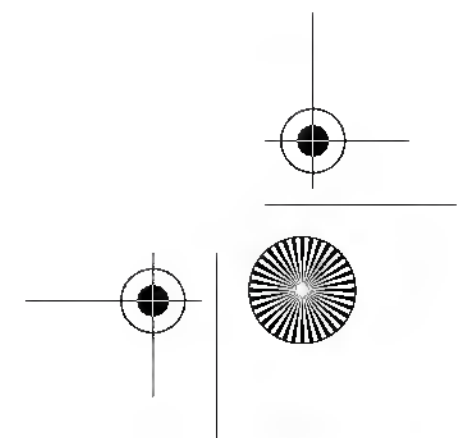
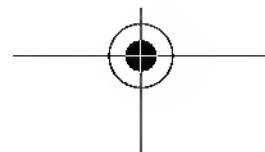
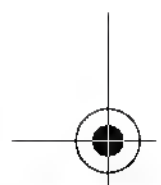
For spoilage bacteria from chilled foods, growth kinetics at low temperatures are often well characterized but values of μ_{\max} , μ_{opt} , T_{opt} , pH_{opt} , and pH_{\max} are frequently unknown or have not been determined accurately. This is the case, for example, for the specific spoilage organisms *Photobacterium phosphoreum*, *Shewanella putrefaciens*, and *Brochothrix thermosphacta*. In this situation the classical gamma concept cannot be used to develop a secondary model. However, when a simple square-root-type model including the effect of temperature and, e.g., CO_2 , has been developed for chilled product stored at a known pH (pH_{ref}) and water activity ($a_{w \text{ref}}$) then these models can be expanded at suboptimal growth conditions by addition of γ -like factors, as shown in Equation 3.18 (Dalgaard et al., 2003).

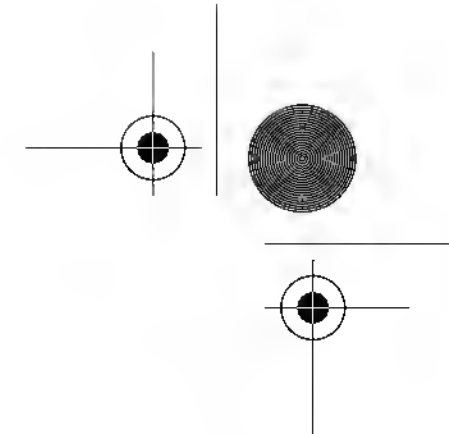
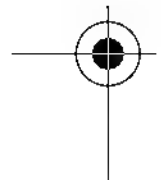
$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}) \cdot (\% \text{CO}_2_{\max} - \% \text{CO}_2) / \% \text{CO}_2_{\max} \cdot \sqrt{(a_w - a_{w \min}) / (a_{w \text{ref}} - a_{w \min})} \cdot \sqrt{(\text{pH} - \text{pH}_{\min}) / (\text{pH}_{\text{ref}} - \text{pH}_{\min})} \quad (3.18)$$

Clearly, this approach should be used with some caution because the assumption of independent action has not been tested for all environmental factor combinations. Thus, the range of applicability of the expanded model should be evaluated, e.g., by comparison with data from challenge tests or naturally contaminated products (Giménez and Dalgaard, in press). (Section 3.2.5 discusses the expansion of existing polynomial models.)

3.2.3 CARDINAL PARAMETER MODELS

Cardinal parameter models (CPMs) were introduced to predictive microbiology in 1993 and have become an important group of empirical secondary models (Augustin and Carlier, 2000a,b; Le Marc et al., 2002; Messens et al., 2002; Pouillot et al., 2003; Rosso, 1995, 1999; Rosso et al., 1993, 1995; Rosso and Robinson, 2001). The basic idea behind CPMs is to use model parameters that have a biological or graphical interpretation. When models are fitted to experimental data by nonlinear regression (see Chapter 4), this has the obvious advantage that appropriate starting values are easy to determine. General CPMs rely on the assumption that the inhibitory effect of environmental factors is multiplicative, an assumption that was





formalized in the gamma (γ) concept discussed above (Section 3.2.2). Thus, general CPMs consist of a discrete term for each environmental factor, with each term expressed as the growth rate *relative to that when that factor is optimal*; i.e., each term has a numerical value between 0 and 1. At optimal growth conditions all terms have a value of 1 and thus μ_{\max} is equal to μ_{opt} (Equation 3.19).

Equation 3.19 to Equation 3.21 show a CPM that includes the effect of temperature (T), water activity (a_w), pH, inhibitory substances (c_i) and qualitative factors (k_j) on μ_{\max} (Augustin and Carrier, 2000a). This extensive CPM was developed from available literature data from many studies for growth of *Listeria monocytogenes*. The inhibitory substances included (1) undissociated acetic acid, lactic acid, and citric acid, (2) Na-benzoate, K-sorbate, and the undissociated form of sodium nitrite, and (3) glycerol monolaurin, butylated hydroxyanisole, butylated hydroxytoluene, *tert*-butylhydroquinone, CO_2 , caffeine, and phenol. In addition, the effect of competitive growth of microorganisms and the inhibitory effect due to specific types of foods were included in the model as qualitative factors.

$$\mu_{\max} = \mu_{\text{opt}} \cdot CM_2(T) \cdot CM_2(a_w) \cdot CM_1(\text{pH}) \cdot \prod_{i=1}^n \gamma(c_i) \cdot \prod_{j=1}^p k_j \quad (3.19)$$

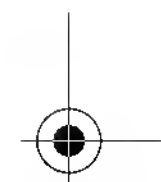
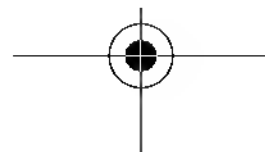
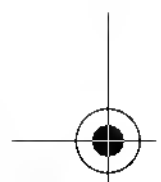
$$CM_n = \begin{cases} 0, & X \leq X_{\min} \\ \frac{(X - X_{\max}) \cdot (X - X_{\min})^n}{(X_{\text{opt}} - X_{\min})^{n-1} \cdot [(X_{\text{opt}} - X_{\min}) \cdot (X - X_{\text{opt}}) - (X_{\text{opt}} - X_{\max})] \cdot ((n-1) \cdot X_{\text{opt}} + X_{\min} - n \cdot X)}, & X_{\min} < X < X_{\max} \\ 0, & X \geq X_{\max} \end{cases} \quad (3.20)$$

$$\gamma(c_i) = \begin{cases} (1 - c_i / MIC_i)^2, & c_i < MIC_i \\ 0, & c_i \geq MIC_i \end{cases} \quad (3.21)$$

where X is temperature, water activity, or pH. X_{\min} and X_{\max} are, respectively, the values of X_i below and above which no growth occurs, X_{opt} is the value at which μ_{\max} is equal to its optimal value μ_{opt} . MIC_i is the minimal inhibitory concentration of specific compounds above which no growth occurs.

Within predictive microbiology various CPMs were developed during the 1990s and in the same period different cardinal parameter temperature models were independently developed in other fields, e.g., to predict the effect of temperature on growth rates (r) of crops (Equation 3.22; Yan and Hunt, 1999; Yin and Wallace, 1995).

$$r = r_{\max} \left(\frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}} \right) \left(\frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}} \right)^{\frac{T_{\max} - T_{\text{opt}}}{T_{\text{opt}} - T_{\min}}} \quad (3.22)$$



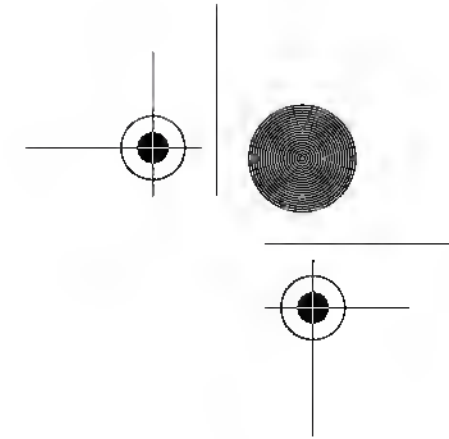
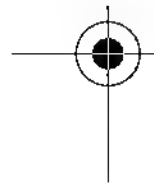


TABLE 3.2
Parameter Values in Square-Root Type (Sqrt) and Cardinal Parameter Models (CPM)

| Organism | T_{min} | | T_{opt} | | T_{max} | | μ_{opt} | | Reference |
|-------------------------------|-----------|-----|-----------|------|-----------|------|-------------|-----|---------------------|
| | Sqrt | CPM | Sqrt | CPM | Sqrt | CPM | Sqrt | CPM | |
| <i>Escherichia coli</i> | 2.9 | 4.9 | 41.0 | 41.3 | 49.2 | 47.5 | 2.3 | 2.3 | Rosso et al. (1993) |
| <i>Salmonella</i> Typhimurium | 3.8 | 5.7 | 39.8 | 40.0 | 51.1 | 49.3 | 1.7 | 1.7 | Oscar (2002) |

| | pH_{min} | | pH_{opt} | | pH_{max} | | μ_{opt} | | Reference |
|-------------------------------|------------|-----|------------|-----|------------|-----|-------------|------|---------------------|
| | Sqrt | CPM | Sqrt | CPM | Sqrt | CPM | Sqrt | CPM | |
| <i>Listeria monocytogenes</i> | 4.2 | 4.6 | 7.0 | 7.1 | 9.8 | 9.4 | 1.0 | 0.95 | Rosso et al. (1995) |

In several ways CPMs resemble square-root models and responses of the two types of models can be practically identical, e.g., for the effect of temperature, water activity, and pH (Oscar, 2002; Rosso et al., 1993, 1995). Parameters in the two types of models are typically named T_{min} , T_{max} , $a_{w\ min}$, $a_{w\ max}$, pH_{min} , and pH_{max} . However, these model parameters are not defined in entirely the same way for CPMs and square-root-type models. In fact, when identical data are fitted to the two types of models square-root-type models estimate lower T_{min} , $a_{w\ min}$, and pH_{min} values and higher T_{max} , $a_{w\ max}$, and pH_{max} values (Table 3.2; see also Chapter 4).

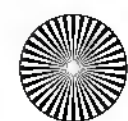
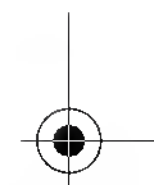
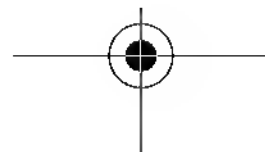
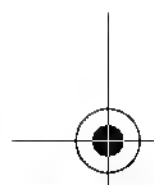
T_{min} values estimated by CPMs and square-root-type models often differ by $\sim 2^\circ\text{C}$ as shown in Table 3.2. Table 3.3 shows that a 2°C difference of a T_{min} value has a pronounced effect on μ_{max} values predicted by both a square-root-type model and a CPM. Thus, parameter values estimated by using one of these types of models

TABLE 3.3
Effect of T_{min} Values (-1°C and $+1^\circ\text{C}$) on μ_{max} Values Predicted by a Square-Root and a Cardinal Parameter Model at 4, 8, and 12°C

| Temperature ($^\circ\text{C}$) | Square-Root Model ^a | | | Cardinal Parameter Model ^b | | |
|-------------------------------------|---------------------------------|------------------------------|------------|---------------------------------------|------------------------------|------------|
| | μ_{max} (h^{-1}) | | % | μ_{max} (h^{-1}) | | % |
| | $T_{min} = -1^\circ\text{C}$ | $T_{min} = +1^\circ\text{C}$ | Difference | $T_{min} = -1^\circ\text{C}$ | $T_{min} = +1^\circ\text{C}$ | Difference |
| 4 | 0.0216 | 0.0078 | 64 | 0.0216 | 0.0087 | 60 |
| 8 | 0.0700 | 0.0424 | 40 | 0.0718 | 0.0485 | 33 |
| 12 | 0.1461 | 0.1046 | 28 | 0.1549 | 0.1237 | 20 |

^a The model of Ratkowsky et al. (1983) used with values of the model parameters b and c selected to obtain a T_{opt} value $\sim 37^\circ\text{C}$ and a μ_{opt} value of $\sim 1.0\ \text{h}^{-1}$. T_{max} was 45.0°C .

^b The model of Rosso et al. (1993) used with T_{opt} of 37°C , μ_{opt} $1.0\ \text{h}^{-1}$, and T_{max} 45.0°C .



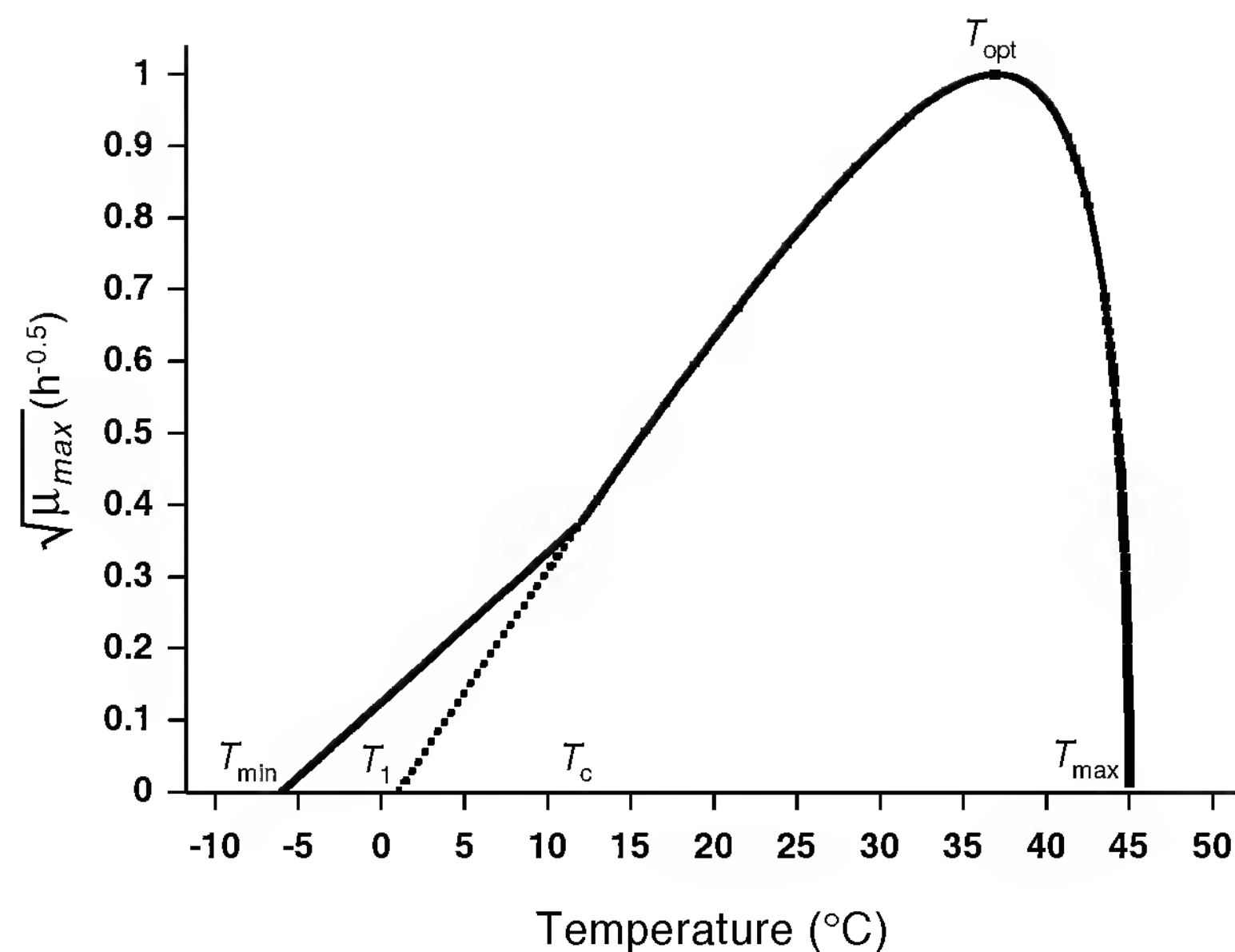
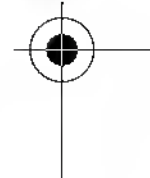
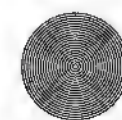
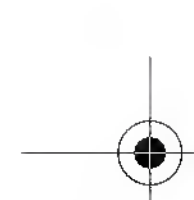
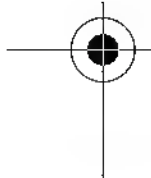


FIGURE 3.3 Simulation of the model $\mu_{\max} = \mu_{\text{opt}} \times \text{CM}_2(T)$, with $\text{CM}_2(T)$ given by Equation 3.23 and with T_{\min} of -6°C , T_1 of 1°C , T_c of 12°C , T_{opt} of 37°C , and T_{\max} of 45°C . μ_{opt} was 1.0 h^{-1} .

cannot be used with the other type of model. This situation is similar to the estimation of μ_{\max} values by some primary growth models. Modified Gompertz models (Gibson et al., 1987; Zwietering et al., 1990), e.g., overestimate μ_{\max} by $\sim 15\%$ (Dalgaard et al., 1994; Membré et al., 1999; Whiting and Cygnarowicz-Provost, 1992) and their growth rate values should not be used together with the exponential, the logistic, or other Richards family of growth models that rely on accurate μ_{\max} values.

Classical CPMs (Equation 3.19 and Equation 3.20) as well as square-root-type models describe a straight line relation between suboptimal temperatures and $\sqrt{\mu_{\max}}$ (Figure 3.1 and Figure 4.4 [Chapter 4]). It has been reported by Bajard et al. (1996) that a different, biphasic, relationship can be observed for some strains of *Listeria monocytogenes*. More recently, Le Marc et al. (2002) observed a biphasic relationship for a strain of *Listeria innocua*. Le Marc et al. (2002) suggested an expanded CPM (Equation 3.23) to simulate this type of growth response (Figure 3.3). In Equation 3.23, T_c is the change temperature and T_1 corresponds to the T_{\min} value in a classical cardinal temperature model (Rosso et al., 1993). McMeekin et al. (1993), however, cautioned against the interpretation of apparently continuously curved relationships as the combination of two linear responses, and provided a simple illustration of the effect. It should also be noted that other workers (e.g., Nichols et al., 2002a,b; see also Chapter 4) have not observed the “curvature” in the low temperature region of *L. monocytogenes* growth.



$$CM_2(T) =$$

$$\begin{cases} \frac{(T - T_1)^2 \cdot (T - T_{\max})}{(T_{opt} - T_1) \cdot [(T_{opt} - T_1) \cdot (T - T_{opt}) - (T_{opt} - T_{\max}) \cdot (T_{opt} + T_1 - 2 \cdot T)]} & , T_c < T < T_{\max} \\ \frac{(T_c - T_1)^2 \cdot (T - T_{\max})}{(T_{opt} - T_1) \cdot [(T_{opt} - T_1) \cdot (T_c - T_{opt}) - (T_{opt} - T_{\max}) \cdot (T_{opt} + T_1 - 2 \cdot T_c)]} \cdot \left(\frac{T - T_{\min}}{T_c - T_{\min}}\right)^2 & , T_{\min} < T < T_c \end{cases} \quad (3.23)$$

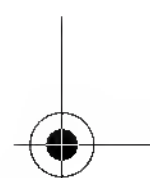
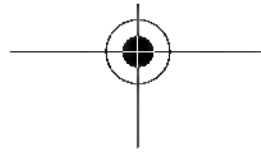
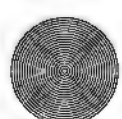
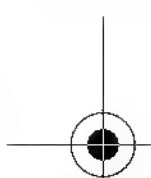
As stated above, general CPMs rely on the assumption that different environmental parameters have independent and thereby multiplicative effects on μ_{\max} (Equation 3.19). The successful use of many general CPMs and square-root-type models has shown this assumption to be reasonable for wide ranges of environmental conditions. However, numerous studies have shown that the growth range of a microorganism to one environmental condition is affected by other environmental factors (see Section 3.4). This suggests that the predictive accuracy of general CPMs can be improved by taking into account interactions between environmental parameters, particularly where one factor is sufficiently stringent that it reduces the growth range of the organism in other environmental “dimensions.”

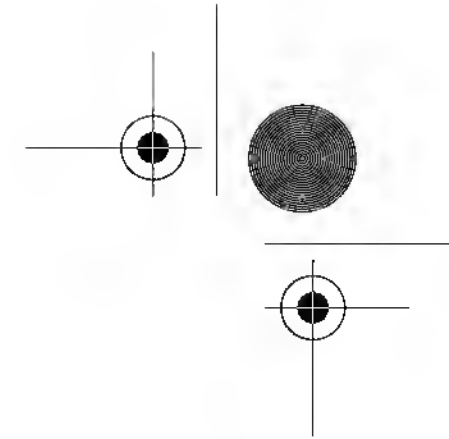
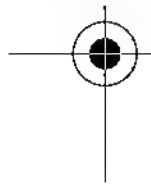
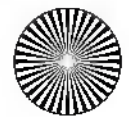
Various approaches have been suggested to describe growth limits under the influence of multiple variables (see Section 3.4.4). Two such approaches have been suggested for direct incorporation in CPMs and are discussed briefly here. Augustin and Carlier (2000b) developed a global secondary model for *L. monocytogenes*, including terms for interactions that prevented growth. Absolute minimal cardinal values X_{\min}^0 were estimated by assuming that all inhibitory substances were absent. Similarly, absolute minimal inhibitory concentrations MIC_i^0 were estimated for optimal concentrations of other environmental parameters ($X = X_{opt}$). Then, interaction between environmental parameters was taken into account by modifying each of the X_{\min}^0 values (Equation 3.24) and the MIC_i^0 values, depending on levels of other environmental parameters. After calculation of appropriate T_{\min} , $a_{w \min}$, pH_{\min} , and MIC_i values, growth rates were then predicted by using Equation 3.19 to Equation 3.21.

$$X_{\min} = X_{opt} - (X_{opt} - X_{\min}^0) \cdot \left(\prod_{i=1}^n \left(1 - \frac{c_i}{MIC_i^0} \right) - \left(\frac{Y_{opt} - Y}{Y_{opt} - Y_{\min}^0} \right)^3 - \left(\frac{Z_{opt} - Z}{Z_{opt} - Z_{\min}^0} \right)^3 \right)^{1/3} \quad (3.24)$$

with X , Y , and Z being temperature, pH, or water activity.

A different approach was used by Le Marc et al. (2002) to model the interactive effects of temperature, pH, and concentration of undissociated organic acids (HA) on growth of *Listeria innocua*. Cardinal parameter values were kept constant and the space of environmental factors was divided into (1) the independent effect space ($\xi = 1$), (2) the interaction space ($0 < \xi < 1$), and (3) the no growth space ($\xi = 0$) (Equation 3.25 to Equation 3.27).





$$\mu_{\max} = \mu_{opt} \cdot CM_2(T) \cdot CM_1(pH) \cdot \tau([HA]) \cdot \xi(T, pH, [HA]) \quad (3.25)$$

$$\xi(\varphi(T, pH, ULA)) = \begin{cases} 1 & \psi \leq \theta \\ 2(1 - \psi) & \theta < \psi < 1 \\ 0 & \psi \geq 1 \end{cases} \quad (3.26)$$

$$\psi = \sum_i \frac{\varphi_{e_i}}{2 \prod_{j \neq i} (1 - \varphi_{e_j})} \quad (3.27)$$

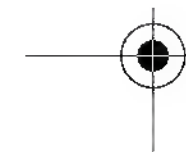
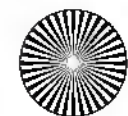
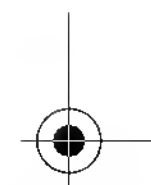
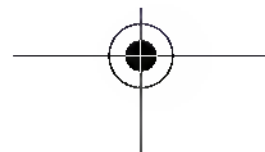
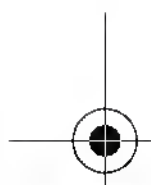
with $\varphi_T = (1 - \sqrt{CM_2(T)})^2$, $\varphi_{pH} = (1 - CM_1(pH))^2$, and $\varphi_{Undissociated\ Lactic\ Acid\ (ULA)} = 1 - (ULA/MIC_{ULA})$ and where e_i are the environmental factors. For calculation of $CM_2(T)$ and $CM_1(pH)$, see Equation 3.20. Le Marc et al. (2002) selected a value of 0.5, which was used for θ .

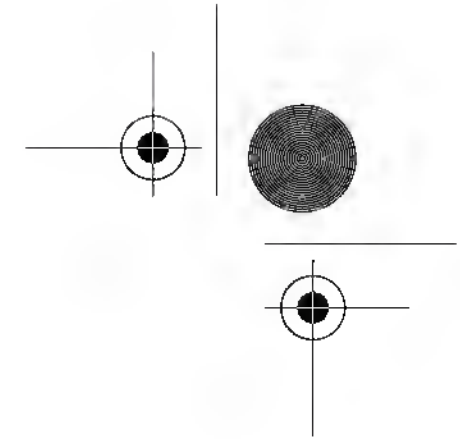
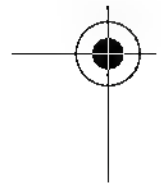
The performance of the two approaches to model interaction between environmental parameters is considered in greater detail in Section 3.4.4. As shown above, CPMs that take into account the effect of interaction between environmental parameters are relatively complicated models. Thus, these models are not fully in agreement with the originally cardinal parameter modeling approach, i.e., that CPM uses only simple biological meaningful parameters that microbiologists are familiar with and that are easy to use by biologists (Rosso et al., 1993), and raises questions about whether those models are the most parsimonious forms available.

The model suggested by Augustin and Carlier (2000b) predicts the effect of interaction between temperature, pH, and lactic acid concentration on growth of *Listeria monocytogenes* to be more pronounced than the effect predicted for *Listeria innocua* by the model of Le Marc et al. (2002). For example, the Augustin and Carlier (2000b) model predicts no growth of *Listeria monocytogenes* at 8°C, pH 6.0, and with 200 mM of lactic acid, whereas at this condition the model of Le Marc et al. (2002) predicts growth and also that there is no interactive effect of the environmental factors ($\xi = 1$). Recently, Giménez and Dalgaard (in press) found the model of Augustin and Carlier (2000b) to substantially underestimate growth of *Listeria monocytogenes* in cold-smoked salmon. This could indicate that the model is in fact overestimating the importance of the interaction between at least some sets of environmental factors.

In a similar vein Ratkowsky and Ross (1995), recognizing the relationship between absolute limits for each environmental factor and their relationship to the parameters of square-root-type models and CPMs, experimented with the use of a kinetic model as the basis of a growth boundary model using linear logistic regression. This approach is discussed later (see Section 3.4.3.2).

The classical CPMs, in particular those including the effect of temperature, water activity, or pH, are now popular and used for many purposes within predictive microbiology (see Table 3.5 and Table 3.6). As one example a cardinal temperature and pH model has been combined with classical models of microbial kinetics, i.e.,





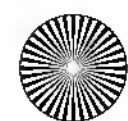
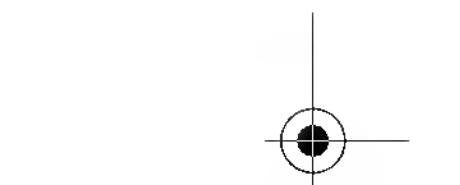
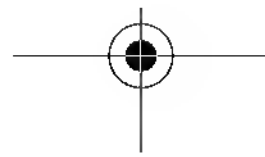
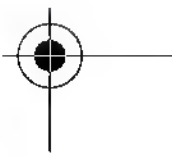
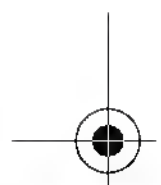
models that rely on yield factors and maintenance constants. In this way, production of curvacin A by *Lactobacillus curvatus* LTH 1174 growing in MRS broth was successfully modeled between 20 and 38°C and at pH values from 4.8 to 7.0 (Messens et al., 2002). Other examples include the use for CPMs to predict the radial growth rate of molds on solidified laboratory media (Panagou et al., 2003; Rosso and Robinson, 2001; Sautour et al., 2001). The ability of these models to predict growth in foods deserves further study.

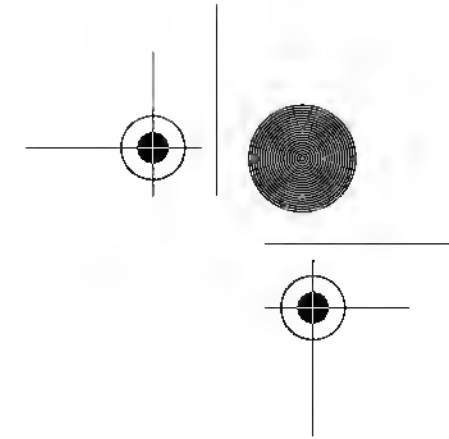
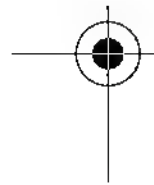
For practical use of secondary predictive models it is important to know the precision of the predicted responses. With CPMs it has been suggested to determine cardinal parameters values for a number of different strains within each of the microbial species of interest (Membré et al., 2002). In this way a measure of intra-species variability can be obtained. As an example, variability in the pH_{min} value for 10 strains of *E. coli* was ± 0.20 corresponding to approximately four times the experimental error (Membré et al., 2002). More recently Pouillot et al. (2003) suggested the use of a CPM together with a Bayesian procedure for parameter estimation. This approach includes the use of hyperparameters and allows uncertainty (due to imperfect knowledge or data) and true variability (e.g., due to difference between strains) to be determined separately (see also [Chapter 4](#)). The approach seems most interesting and definitely deserves to be studied further for different secondary predictive models.

3.2.3.1 Secondary Lag Time Models and the Concept of Relative Lag Time

When exponentially growing microorganisms are transferred from one environment into another, similar environment, growth usually continues without delay, i.e., a lag time is rarely observed. However, when the two environments differ, a lag time is often observed. Similarly, when microorganisms in the lag or stationary phases are transferred into identical or new environmental conditions a lag time may continue or result, respectively. Depending on the physiological state of the microorganisms, the magnitude of the shift in the environmental conditions, and the new environmental conditions themselves, the duration of the lag time may range from 0 to infinity.

Development of secondary lag time models is complicated by the fact that lag time is influenced not only by the actual environmental conditions but also by previous environmental conditions and the physiological status of the cell, i.e., the growth phase of microorganisms at the time of transfer between environments and their “enzymatic readiness” to exploit the specific carbon and energy resources within the new environment. Within predictive microbiology, two main approaches have been used for development of secondary lag time models: (1) models where lag time and growth rate are modeled independently and (2) models where lag time is assumed proportional to the generation time. The latter group of models typically rely on the assumption that microorganisms need to perform a given amount of work to adapt to a new environment and that the rate at which this work can be done depends on the growth rate potential of the organism in the new environment (Robinson et al., 1998).





In the former approach, lag times or lag rates (i.e., reciprocal of lag time) are typically log-transformed to stabilize the variance of these data. Frequently, polynomial models (see Section 3.2.5) or artificial neural networks (see Section 3.2.6) have been used to develop independent secondary lag time models (Table 3.5). To model the effect of temperature downshifts, temperature upshifts, and physiological status of cells (e.g., exponential phase, stationary phase, starved, frozen, dried), separate polynomial models have been used for the different physiological conditions (Whiting and Bagi, 2002). When square-root-type and Arrhenius-type models are used for lag time modeling, lag rates are modeled or reciprocal forms of the growth rate models are used (see Section 3.2.1 and Section 3.2.4; Table 3.5 and Table 3.6).

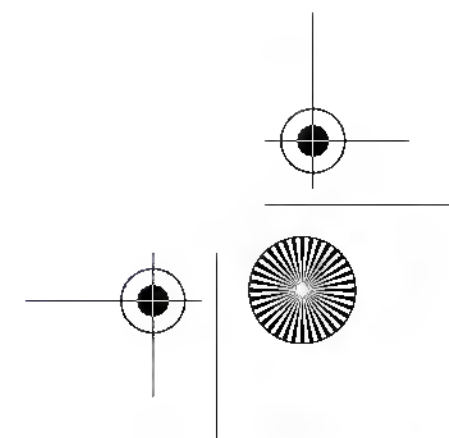
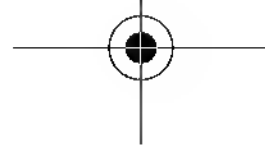
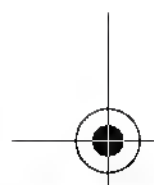
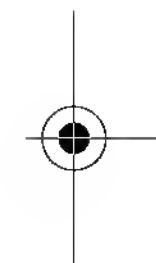
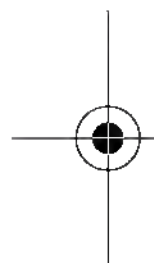
Zwietering et al. (1994), e.g., used a square-root model (Equation 3.2) with identical values of the parameters T_{\min} , c , and T_{\max} to model lag time and growth rate — only the value of b differed between the two models. Specific secondary lag time models for particular environmental parameters have also been suggested, e.g., a hyperbola model for the effect of temperature (Equation 3.28; Oscar, 2002; Zwietering et al., 1994):

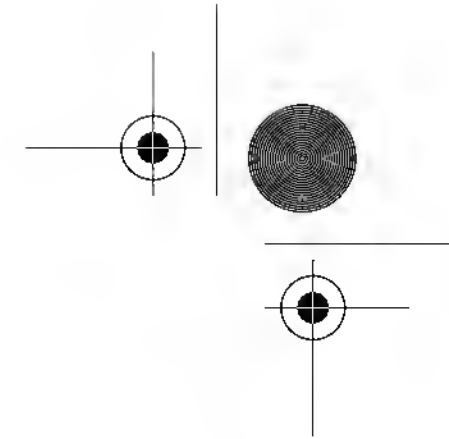
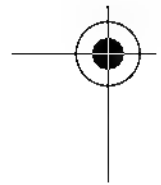
$$\lambda = \left[\frac{p}{T - q} \right]^m \quad (3.28)$$

where λ is the lag time, T the temperature, p the rate of change of lag time as a function of temperature, q the temperature at which lag time is infinite, and m is an exponent to be estimated.

Baranyi and Roberts (1994), Smith (1985), and McMeekin et al. (1993) have observed that lag times for identical inocula introduced to (at least some) environmental conditions are inversely proportional to growth rates and thus proportional to generation times (T_{gen}). This generalization has limits, however, as discussed further below and probably is most relevant to changes in environmental temperature. For example, Zwietering et al. (1994) showed that for the effect of temperature on *Lactobacillus plantarum* the product of μ_{\max} and lag time (λ) was constant and had an average value close to 2. In these situations secondary lag time models can be derived directly from a growth rate model by using the simple concept of relative lag time (RLT; Equation 3.29) in common use but first defined by Mellefont and Ross (2003). Clearly, RLT reflects the physiological status of microorganisms introduced into a new environment as well as the difference between their actual and their previous environments, and can be interpreted as the amount of work the cell has to do to change its physiology (e.g., enzymes, membrane composition, number of ribosomes) to be able to grow at μ_{\max} in that new environment.

Baranyi and Roberts (1994) suggested a primary model to estimate lag times from microbial growth curves and this model allowed determination of the parameters h_0 , q_0 , and α_0 all of which reflect the physiological state of microorganisms and, thereby, their readiness to grow in a given environment (Equation 3.29; Chapter 2). It can be seen that the parameter RLT is directly proportional to h_0 .





$$\frac{\lambda}{T_{gen}} = RLT, \quad \lambda = \frac{RLT \cdot \ln(2)}{\mu_{max}}, \quad (3.29)$$

$$\lambda \cdot \mu_{max} = RLT \cdot \ln(2) = h_o = \ln\left(1 + \frac{1}{q_o}\right) = -\ln(\alpha_o)$$

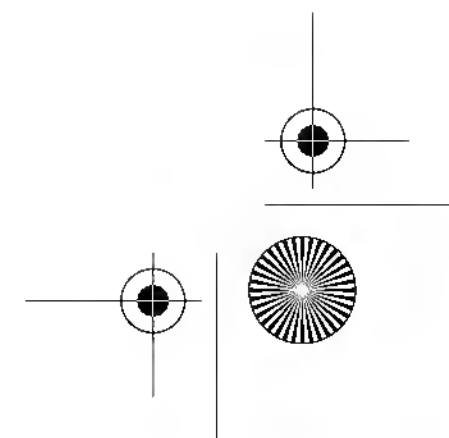
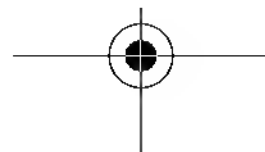
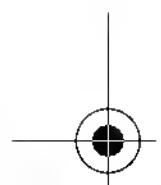
where all parameters have meanings as indicated earlier.

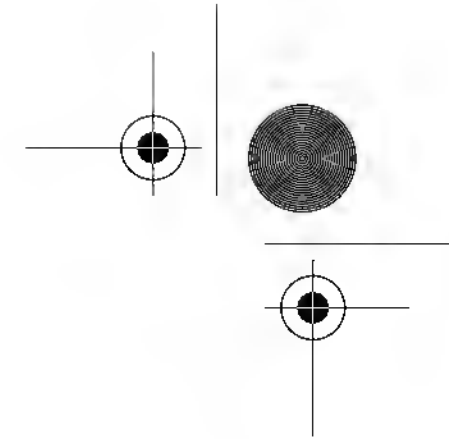
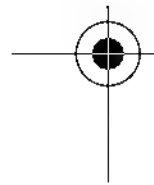
Experimental methods to determine the physiological status of low levels of microorganisms in foods remain to be developed. Thus, for the time being these parameters have mainly theoretical importance.

The RLT concept is practically very useful for development of secondary lag time models, but it should be used with caution. Delignette-Muller (1998) evaluated data from nine studies where the effect of temperature, pH, NaCl, and NaNO₂ on lag time and generation time on different food-borne microorganisms had been modeled independently. In four of the nine studies, RLT was constant and an independent lag time model was not needed. However, primarily pH and NaCl influenced RLT in the remaining studies. On the basis of large amounts of experimental data, Ross (1999a) showed the distribution of RLT of *B. stearootherophilus*, *Clostridium perfringens*, *E. coli*, *L. monocytogenes*, *Salmonella*, and *S. aureus* included peaks in the range 3 to 6 under a very wide range of experimental conditions. These distributions were similar to those presented by Augustin and Carlier (2000a), who observed a median RLT of 3.09 for *L. monocytogenes* ($n = 1176$). Using extreme environmental shifts, and severely growth-limiting outgrowth conditions, the hypothesis that RLT values have an upper limit was tested (Mellefont et al., 2003, in press). It was found that most RLTs were in the range 4 to 6, and that RLTs greater than 8 could not be induced within the experimental system employed. These observations suggest that while lag time is apparently highly variable, RLT is more uniform and reproducible. Distributions of RLT can be used in stochastic modeling studies, for example, microbial food safety risk assessments, where they could be used as plausible default assumptions if specific lag time information was not available. This approach can also simplify the growth modeling process because use of the RLT as a variable enables the effects of growth rate and lag to be predicted by a single growth rate model, as explained above.

The RLT concept implies that λ is at a minimum value (λ_{min}) when the growth rate is optimal (μ_{opt}). This relation has been used together with CPMs to obtain simple secondary lag time models (Equation 3.30 and Equation 3.31; Augustin and Carlier, 2000a; Le Marc et al., 2002; Pouillot et al., 2003; Rosso, 1995, 1999a,b).

$$\lambda = \frac{\lambda_{min} \cdot \mu_{opt}}{\mu_{max}} \quad (3.30)$$

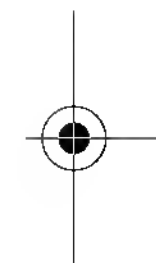
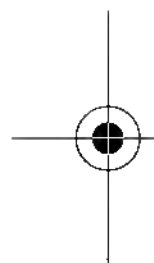




$$\lambda = \frac{\lambda_{\min}}{CM_2(T) \cdot CM_2(a_w) \cdot CM_1(\text{pH})} \quad (3.31)$$

For RLT models to be used in practice it must be known if, and to what extent, abrupt or smooth shifts in environmental parameters like temperature, pH, and water activity influence RLT.

Data presented by Rosso (1999a,b) suggested that the effect of shifts in temperature and pH on growth of *E. coli* during fermentation of yoghurt was appropriately predicted by a CPM that relied on assumption of a constant RLT. Augustin et al. (2000) suggested a model to take into account the effect of growth phase and temperature history of *L. monocytogenes* on its RLT. For temperature downshifts the RLT increased from ~ 0 for a temperature shift of 0–5°C to ~ 2 for a downshift of 30–35°C. To model the effect of temperature downshifts and upshifts on RLT of *L. monocytogenes*, Delignette-Muller et al. (2003) recently used the data of Whiting and Bagi (2002) and suggested simple biphasic linear models. Separate models were used for inoculum with different physiological states. For *E. coli*, Mellefont and Ross (2003) found a similar effect of temperature downshifts whereas temperature upshifts had no systematic effect on RLT. For abrupt downshifts and upshifts in water activity the data of Mellefont et al. (2003) suggest that simple biphasic linear models, with different slopes for down- and upshifts, may be appropriate to predict RLT of both Gram-negative and Gram-positive food-borne bacteria. The universality of these responses remains unclear. For example, RLTs of *S. aureus* and *L. monocytogenes* were largely unaffected by abrupt osmotic shifts over a wide range of salt concentrations, whereas RLT of Gram-negative cells was strongly affected. More research is required before models that are as reliable as existing growth rate models can be developed for lag time, or RLT.



3.2.4 SECONDARY MODELS BASED ON THE ARRHENIUS EQUATION

3.2.4.1 The Arrhenius Equation

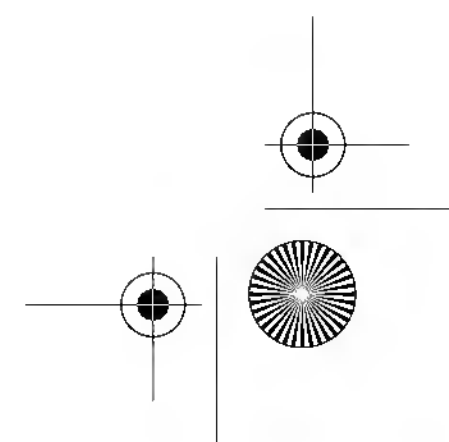
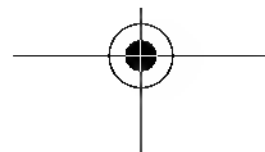
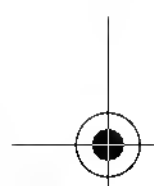
The empirical Arrhenius–van't Hoff relationship:

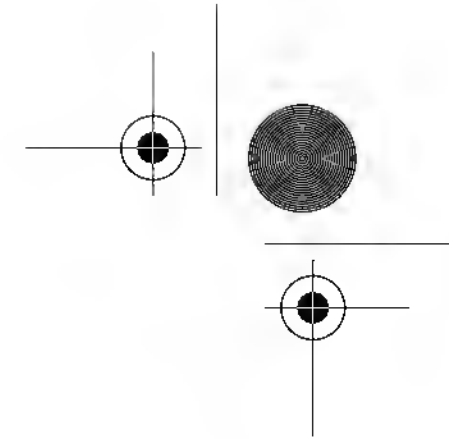
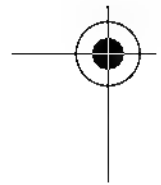
$$\text{rate} = A \exp(\Delta E_a / RT) \quad (3.32)$$

or its mechanistic interpretation and modification due to Eyring (1935) based in absolute reaction-rate theory:

$$\text{rate} = KT \exp(\Delta H^\ddagger / RT) \quad (3.33)$$

where the parameters may be interpreted as follows: A is a constant related to the number of collisions between reactants per unit time, E_a the activation energy, R the gas constant (8.314 J/K/mol), T the temperature in Kelvin, K is similar to A but includes steric and entropic effects, and ΔH^\ddagger is the enthalpy difference between the





transition state complex and the reactants, are well established in chemistry to describe the effects of temperature on the rate of chemical reactions. Taking the logarithm of both sides of Equation 3.32:

$$\ln(\text{rate}) = \ln(A) \times \Delta E/RT$$

and reparameterizing the equation becomes:

$$\ln(\text{rate}) = A' + \left(\frac{\Delta E}{R}\right) \times \left(\frac{1}{T}\right)$$

Thus, if $\ln(\text{rate})$ is plotted against $\left(\frac{1}{T}\right)$, the resulting plot is a straight line over temperature ranges relevant to microbial growth and allows estimation of the “activation energy” of the reaction, as shown in Figure 3.4. The activation energy can be used to characterize the reaction.

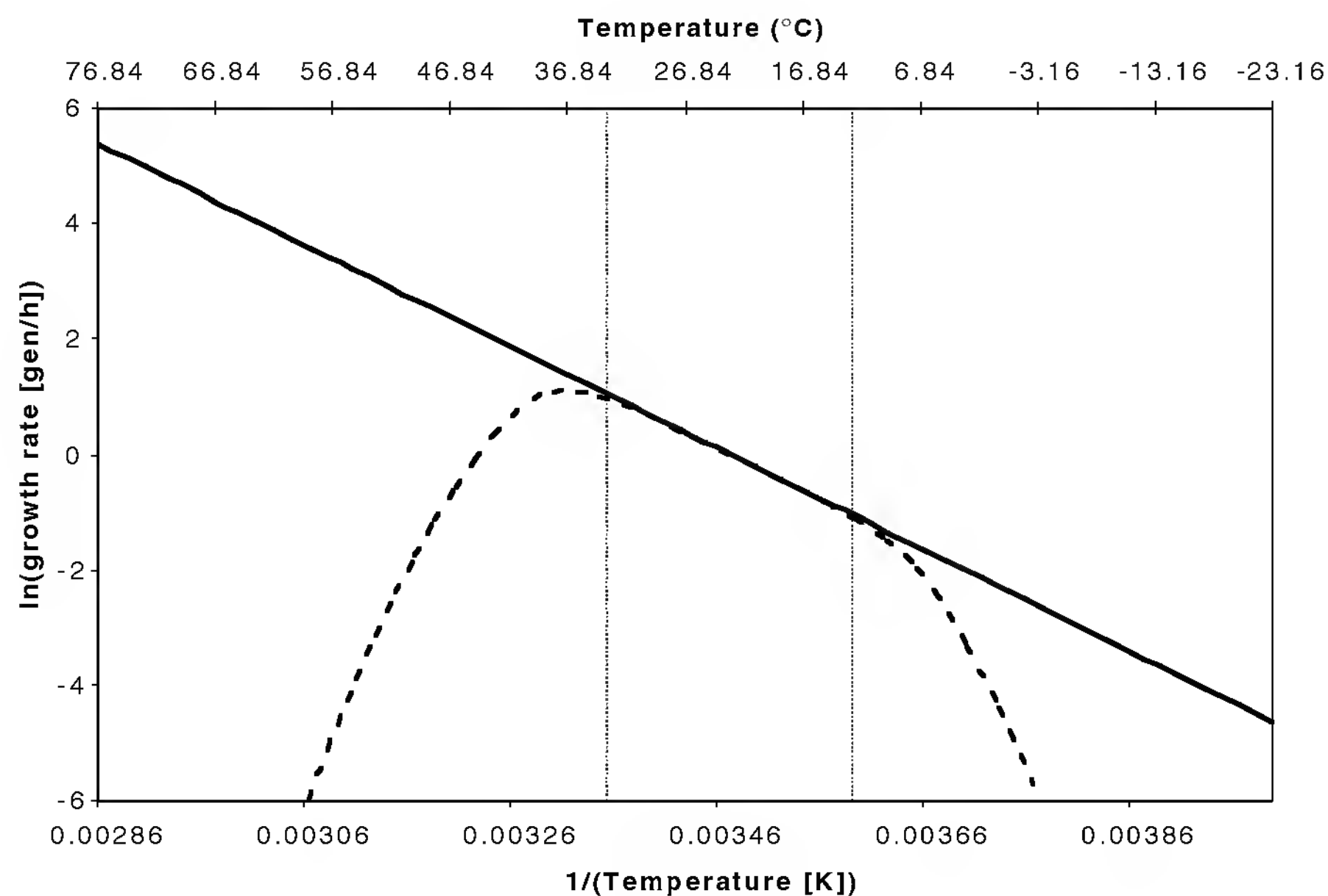
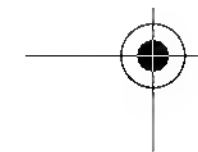
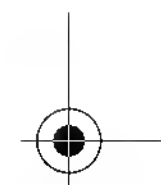
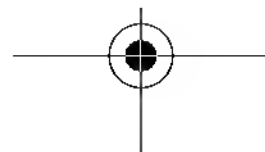
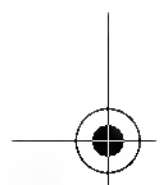
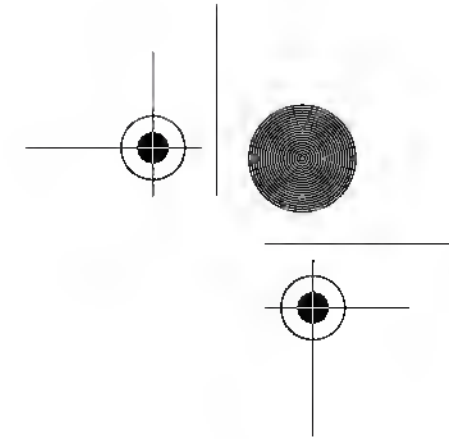
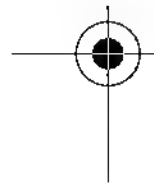


FIGURE 3.4 Diagram showing the effect of temperature on reaction rate predicted using the Arrhenius model (Equation 3.33; solid line) and the effect of temperature on microbial growth rate (dashed line) for a representative mesophilic organism. The “activation energy” is estimated from the slope of the solid line, multiplied by the universal gas constant. Over a narrow range of temperatures, the microbial growth rate follows the Arrhenius model prediction (Equation 3.29). This range has been termed the “normal physiological range” (NPR). At temperatures above or below the NPR, microbial growth rate deviates markedly from that predicted by the Arrhenius model.





It has been argued that because all life processes are the result of chemical reactions, the growth rate of organisms that cannot achieve thermal homeostasis should also be described by Arrhenius kinetics. Within a narrow range of temperature this is true. In practice, however, when microbial growth rate data for the full biokinetic temperature range are presented as an Arrhenius plot, the data are far from linear as shown in [Figure 3.4](#), and confirmed by numerous studies (Heitzer et al., 1991; McMeekin et al., 1993; Schoolfield et al., 1981).

A range of secondary models, based on adherence to the reaction kinetics described by the Arrhenius model, but including terms to account for the observed deviations, have been proposed. These models fall into two groups:

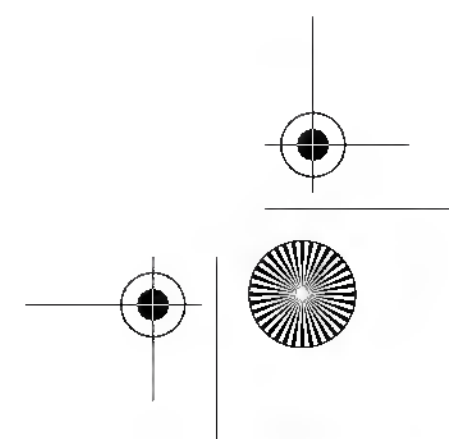
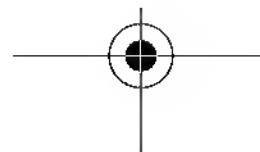
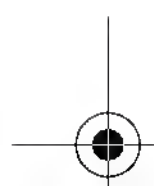
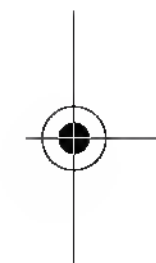
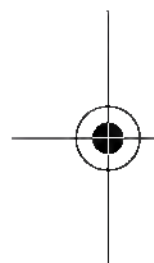
1. Those based on putative mechanistic modifications of the Arrhenius models
2. Those based on empirical modifications

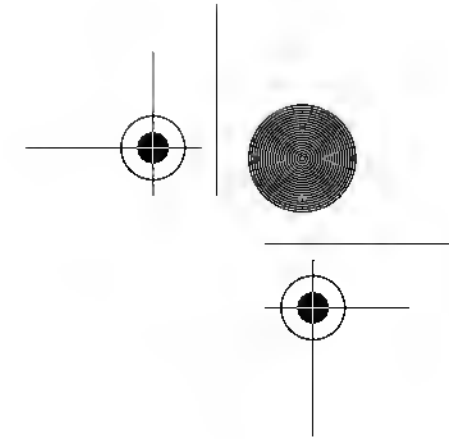
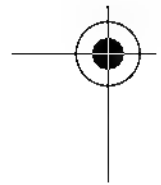
3.2.4.2 Mechanistic Modifications of the Arrhenius Model

Models in this category include those of Johnson and Lewin (1946) to describe the high-temperature growth of bacteria, Hultin (1955) to describe rates of enzymatic catalysis in the low temperature region, Sharpe and DeMichele (1977) who synthesized these two equations to produce a model for the temperature dependence of bacterial growth rate in the entire biokinetic region, the model of Schoolfield et al. (1981), which is a reparameterization of the Sharpe and DeMichele model to overcome difficulties in fitting by nonlinear regression, and the models of McMeekin et al. (1993) and Ross (1993a, 1999b). The latter models incorporate contemporary knowledge of the thermodynamics of protein folding to overcome failures in the Schoolfield et al. model related to unrealistic parameter estimate (Ratkowsky et al., 1991).

The above models were originally developed to provide an interpretation of microbial growth rates or enzyme-catalyzed reaction rates, in response to temperature but their mechanistic basis makes them attractive for use as secondary models.

This class of secondary models have previously been reviewed (McMeekin et al., 1993; Ratkowsky et al., 1991; Ross, 1999b; Ross and McMeekin, 1994). In summary, all of the models are based on the assumption that there is a single, enzyme-catalyzed, rate-limiting reaction in any microorganism. This reaction is characterized by an activation energy, which governs the rate of reaction in response to temperature, according to Arrhenius kinetics. Enzymes are proteins, however, and are themselves subject to the effects of temperature. The functional activity of enzymes is dependent upon their shape, or conformation, but they are flexible — the flexibility being required to achieve their catalytic function. Because temperature affects the bonds in the molecule, if the temperature changes too much, the conformation becomes so disrupted that denaturation takes place, both at high and low temperatures. These denaturation events are reversible, but at high temperatures if the temperature increases sufficiently, irreversible denaturation takes place (Ross, 1999b). Thus, these models include terms to model the probability, as a function of temperature, that the enzyme is in its metabolically active conformation and use this





estimate to modify the predictions of the Arrhenius model. Equation 3.34 to Equation 3.36 are examples of this form of model.

Model of Hinshelwood (1946):

$$\text{rate} = A_1 \exp(-E_a/RT) - A_2 \exp(-E_{a \text{ high}}/RT) \quad (3.34)$$

where R , T , A , and E_a have the same meaning as above. $E_{a \text{ high}}$ is the activation energy of the high-temperature denaturation of the rate-limiting enzyme.

Model of Schoolfield et al. (1981):

$$\frac{1}{K} = \frac{\rho_{(25)} \frac{T}{298} \exp\left\{\frac{H_A}{R} \left(\frac{1}{298} - \frac{1}{T}\right)\right\}}{1 + \exp\left\{\frac{H_L}{R} \left(\frac{1}{T_{1/2L}} - \frac{1}{T}\right)\right\} + \exp\left\{\frac{H_H}{R} \left(\frac{1}{T_{1/2H}} - \frac{1}{T}\right)\right\}} \quad (3.35)$$

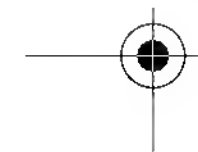
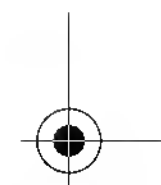
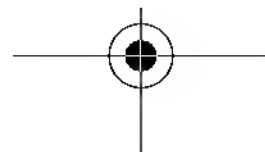
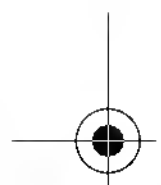
where T is the absolute temperature, R is the universal gas constant, and, for modeling bacterial growth, the other parameters have been interpreted as follows: K is the response (e.g., generation) time, $\rho_{(25)}$ a scaling factor equal to the response rate ($1/K$) at 25°C, H_A the activation energy of the rate-controlling reaction, H_L the activation energy of denaturation of the growth-rate-controlling enzyme at low temperatures, H_H the activation energy of denaturation of the growth-rate-controlling enzyme at high temperatures, $T_{1/2L}$ the lower temperature at which half of the growth-rate-controlling enzyme is denatured, and $T_{1/2H}$ is the higher temperature at which half of the growth-rate-controlling enzyme is denatured.

Model of Ross (1999b):

$$\text{rate} = \frac{CT \exp(\Delta H^\ddagger / RT)}{1 + \exp(-n(\Delta H^* - T\Delta S^* + \Delta C_p[(T - T_H^*) - T \ln(T / T_S^*)]) / RT)} \quad (3.36)$$

where C is a parameter whose value must be estimated, ΔH^\ddagger the activation enthalpy of the reaction catalyzed by the enzyme controlling the overall reaction rate, ΔC_p the difference in heat capacity (per mole amino acid residue) between the native (catalytically active) and denatured state of the enzyme, T_H^* the temperature (K) at which the ΔC_p contribution to enthalpy is 0, T_S^* the temperature (K) at which the ΔC_p contribution to entropy is 0, ΔH^* the value of enthalpy at T_H^* per mole amino acid residue, ΔS^* the value of entropy at T_S^* per mole amino acid residue, T the temperature (K), R the gas constant (8.314 J/K/mol), and n is the number of amino acid residues in the enzyme.

Equation 3.34 to Equation 3.36 include the simple Arrhenius model in the numerator of the equation. The denominators in Equation 3.35 and Equation 3.36, however, model the probability that the enzyme is in its active conformation. When



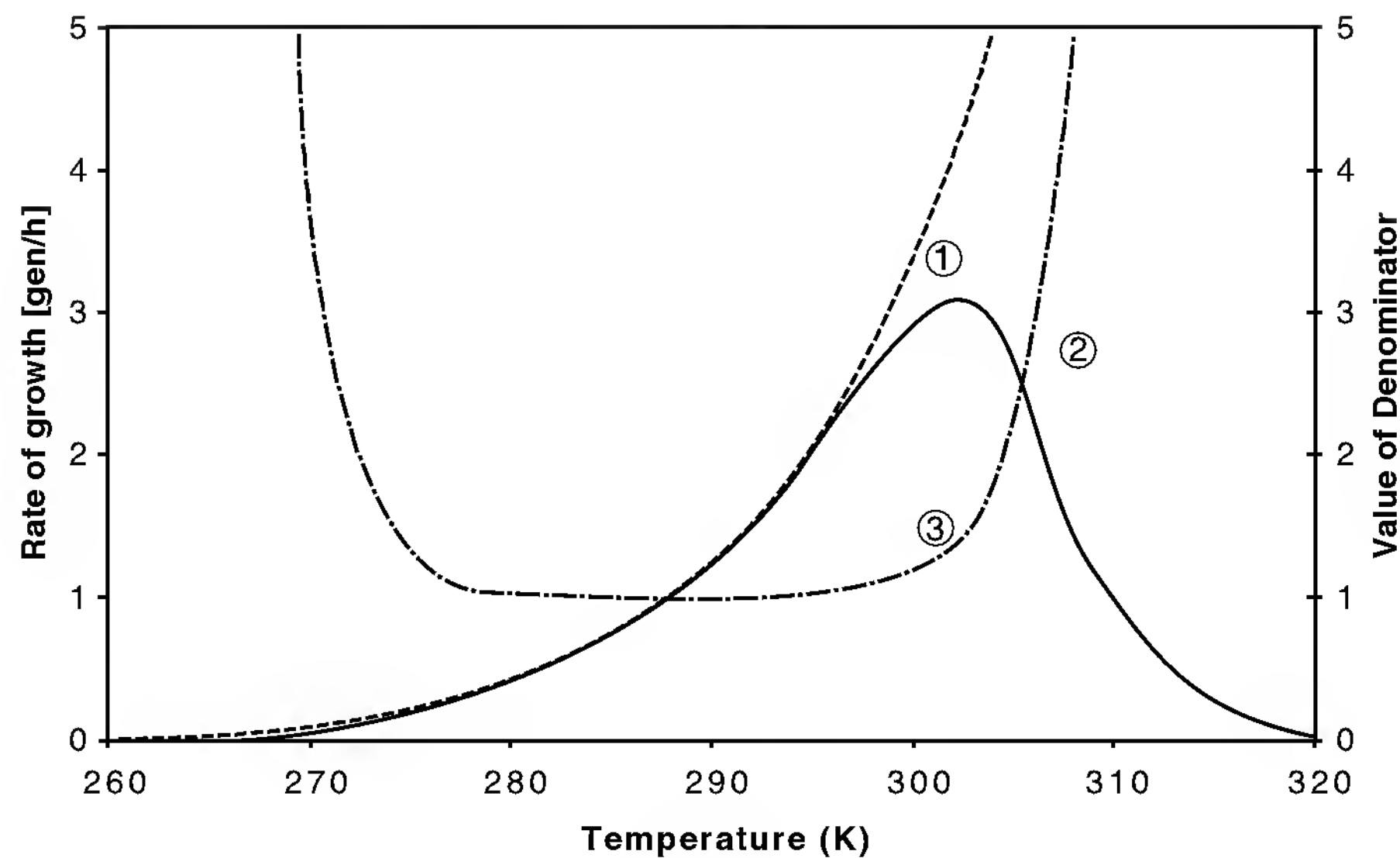
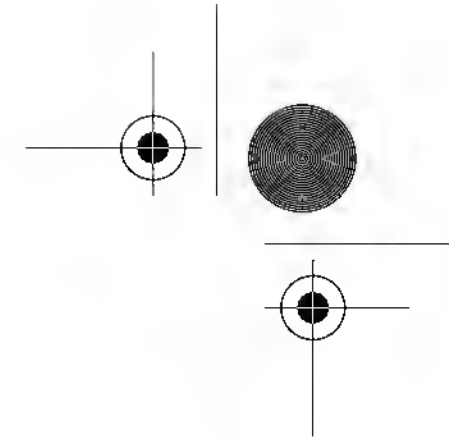
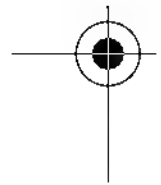
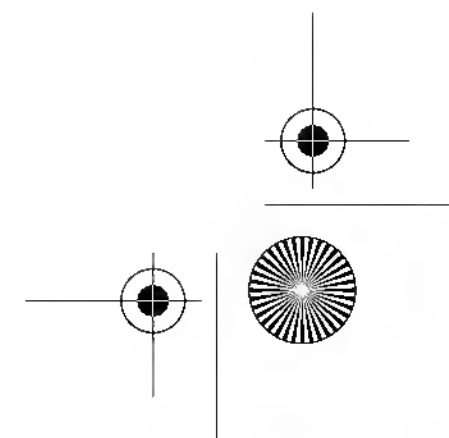
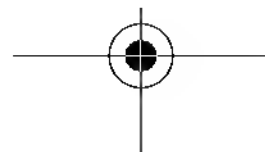
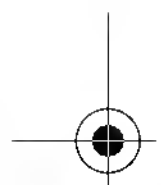
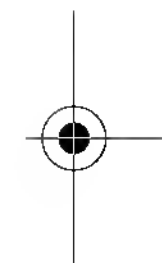
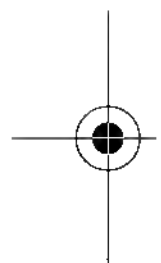
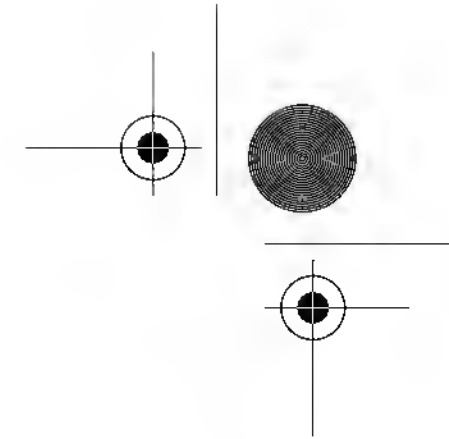
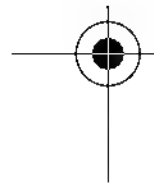


FIGURE 3.5 Diagram showing the interaction of terms in mechanistic models for microbial growth rate response to temperature. Curve 1 (dashed line) is the predicted growth rate in the absence of master enzyme denaturation, i.e., Arrhenius kinetics as modeled by the numerator of Equation 3.36. Curve 2 (dot-dash line) is the inverse of the probability of the “master enzyme” being in the active conformation, i.e., the denominator of Equation 3.36. Curve 3 (solid line) is the overall predicted rate from the model, i.e., the quotient of values in Curve 1 divided by values in Curve 2.

that probability is high, the denominator takes values close to 1, so that the overall rate is close to that predicted by the Arrhenius equation in the numerator. When the probability is lower, the value of the denominator increases, so that the observed rate is lower than that predicted by the numerator alone. These relationships are shown in Figure 3.5 for Equation 3.36, presented as rate vs. temperature for clarity of interpretation.

In practice, few of these types of models have been routinely applied in predictive microbiology, possibly because the models are highly nonlinear, and initial parameter estimates are difficult to determine. Furthermore, it is currently not possible to independently measure the values of the parameters of the model because the putative master reaction has not been identified, and the concept that a single reaction is rate limiting under all environmental conditions seems improbable (Daughtry et al., 1997; Ross, 1999b). Finally, several workers (Heitzer et al., 1991; Ratkowsky, D.A., personal communication, 2003; Ross, 1993b) demonstrated that even with good quality data, square-root-type models provide an equally good fit as those “mechanistic” models, and are usually easier to work with. Examples of their use include Broughall et al. (1983) and Broughall and Brown (1984) who used the Schoolfield model, but also extended it to model the effect of water activity and pH, by replacing some terms in the model with polynomial expressions in a_w and pH. Adair et al. (1989) used a reparameterized form of the Schoolfield et al. model, which was





essentially another form of the Johnson and Lewin (1946) model. Recent studies (Ratkowsky et al., unpublished) have confirmed that Equation 3.36 does describe bacterial temperature–growth rate curves well for a wide range of species, and, in contrast with earlier models, that the estimated parameter values are realistic and consistent with the theoretical bases of the model.

3.2.4.3 Empirical Modifications of the Arrhenius Model

A second class of Arrhenius-based models for growth rate and reciprocal of lag time have been presented by Davey and coworkers. Davey (1989) introduced an Arrhenius-type model for the effects of temperature and water activity, which is linear and thus allows for explicit solution of the optimum parameter values. This model has the form:

$$\ln(\text{rate}) = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_w + C_4 a_w^2 \quad (3.37a)$$

where T is temperature (K), a_w has its usual meaning, and C_0, C_1, C_2, C_3, C_4 are coefficients to be determined.

Davey (1989) reported that the model described well seven data sets from the literature and subsequently demonstrated the ability of the model to also describe the reciprocal of lag phase duration (Davey, 1991). Davey (1994) fitted a variation of the model to the data of Adams et al. (1991) for *Yersinia enterocolitica* growth. The model included terms for temperature and pH, and is analogous to Equation 3.37a:

$$\ln(\text{rate}) = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 pH + C_4 pH^2 \quad (3.37b)$$

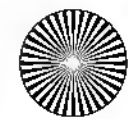
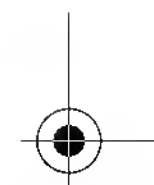
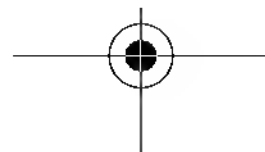
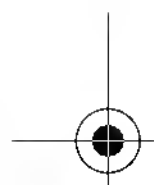
where T is temperature (K), pH has its usual meaning, and C_0, C_1, C_2, C_3, C_4 are coefficients to be determined.

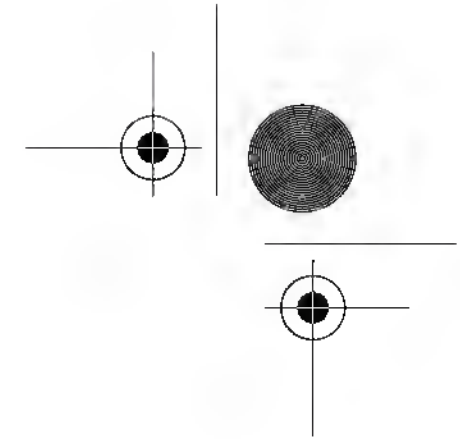
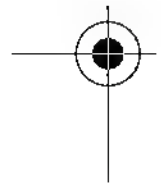
On the basis of these observations, Davey (1994) extended his earlier proposed general model structure for linear Arrhenius models (Davey, 1989) to account for the effect of multiple environmental factors affecting growth rate to the following form:

$$\ln(\text{rate}) = C_0 + \sum_{i=1}^j (C_{2i-1} V_i + C_{2i}^2 V_i) \quad (3.37c)$$

where j environmental factors, V_i act in combination to affect the growth of the modeled organism, and $C_0, C_1, C_2, \dots, C_j$ are coefficients to be determined.

This general form was applied by Davey and Daughtry (1995) to data of Gibson et al. (1988) for *Salmonella* growth in response to temperature, NaCl, and pH. Thus, their equation had the form:





$$\ln(\text{rate}) = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3S + C_4S^2 + C_5pH + C_6pH^2 \quad (3.37d)$$

where S is salt concentration (% w/v).

While the above model forms are empirical, they also recognize implicitly the temperature dependence of microbial growth rates. Daughtry et al. (1997) invoked chemical reaction rate theory to develop an alternative mechanistic model based on the Arrhenius equation. Those workers cited Levenspiel (1972) as stating that curvature in Arrhenius plots can arise if there are two, or more, reactions that “compete” to limit the reaction rate and dominate under different conditions so that the overall effect of temperature is the synthesis of the individual activation energies for the rate-limiting reactions at different temperatures. Daughtry et al. (1997) considered that bacterial growth was likely to be such a system.

By assuming that the “heat of reaction” (equivalent to the activation energy or activation enthalpy in the above discussion) is a polynomial function of temperature, the following modified Arrhenius model was developed:

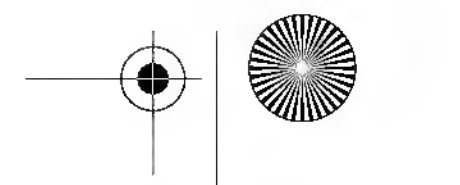
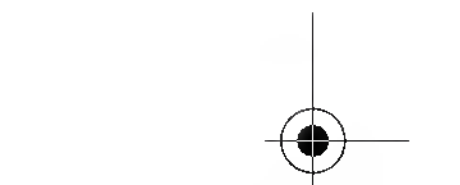
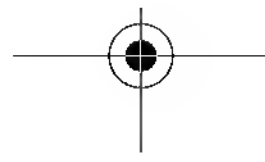
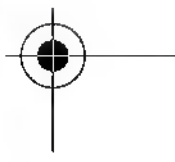
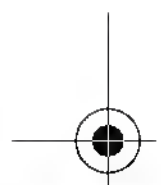
$$\ln(\text{rate}) = C_0 + \frac{C_1}{T} + C_2 \ln T \quad (3.38)$$

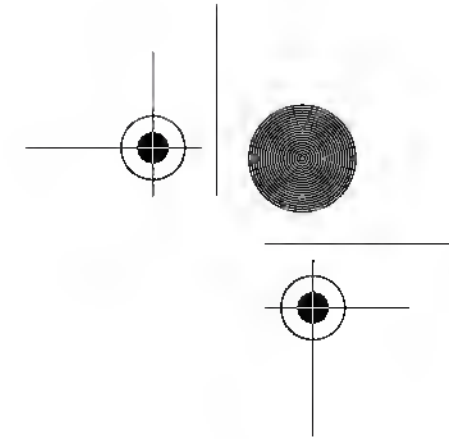
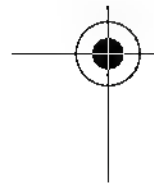
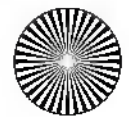
This model fitted experimental data as well as the temperature-only form of Equation 3.37a.

The “linear Arrhenius” or “Davey” models have been used to model growth of molds on solid microbiological media (Molina and Giannuzzi, 1999; Panagou et al., 2003). Panagou et al. (2003) preferred cardinal parameter and gamma-concept-type models (see Sections 3.2.2 and 3.2.3) over the Davey model because of their interpretable parameter values. Davey models have also been applied to UV and thermal inactivation and data describing the combined effects of pH and water activity on thermal inactivation, including vitamin denaturation (see Section 3.3), but they have not been widely adopted by other workers. McMeekin et al. (1993) and Davey (2001) identified a close correlation between estimates of coefficients C_1 and C_2 , and C_3 and C_4 , of Equation 3.37a, suggesting that the model was overparameterized.

3.2.4.4 Application of the Simple Arrhenius Model

For the entire biokinetic temperature range, growth rates of microorganisms are described less appropriately by the Arrhenius-type equations (Equation 3.34 to Equation 3.36) than by square-root-type and cardinal parameter models (see Section 3.2.4.2; Rosso et al., 1993; Zwietering et al., 1991). However, Arrhenius-type models remain useful as secondary kinetic models for less extensive ranges of storage temperatures (Table 3.5 and Table 3.6). Koutsoumanis and Nychas (2000) used Equation 3.32 to model the effect of temperatures between 0 and 15°C on μ_{\max} and reciprocal lag time of naturally occurring pseudomonads growing aerobically on a type of Mediterranean fish. Koutsoumanis et al. (2000) also expanded the classical





Arrhenius model to take into account the combined effect of temperature and CO₂ on growth rates of spoilage bacteria in modified atmosphere packed fresh fish (Equation 3.39).

$$\ln(\mu_{\max}) = \frac{E_a}{R} \times \left(\frac{1}{T_{\text{ref}}} - \frac{1}{T} \right) + \ln(\mu_{\text{ref}} - d_{\text{CO}_2} \times \% \text{CO}_2) \quad (3.39)$$

where T , E_a , and R have their usual meaning, %CO₂ is the equilibrium concentration of CO₂ in the headspace gas, d_{CO_2} is a constant expressing the effect of CO₂ on μ_{\max} and T_{ref} and μ_{ref} are temperature and maximum specific growth rate, respectively, at 273 K and 0 %CO₂. The term including d_{CO_2} in Equation 3.39, describing CO₂ inhibition of growth rate, was previously suggested by Kalina (1993).

The simple Arrhenius model has also been used to calculate relative rates of spoilage (RRS) (Equation 3.37). RRS for a food product is defined as the shelf life (determined by sensory evaluation) at a reference temperature (T_{ref}) divided by the shelf life observed at the actual storage temperature (Equation 3.40).

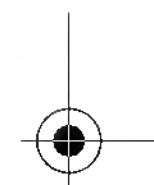
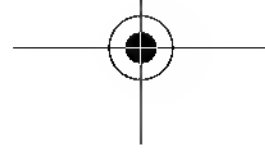
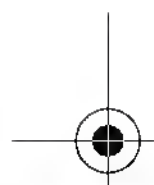
$$RRS = \frac{\text{Shelf life at } T_{\text{ref}}}{\text{Shelf life at } T} = \exp \left[\frac{E_A}{R} \times \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}} \right) \right] \quad (3.40)$$

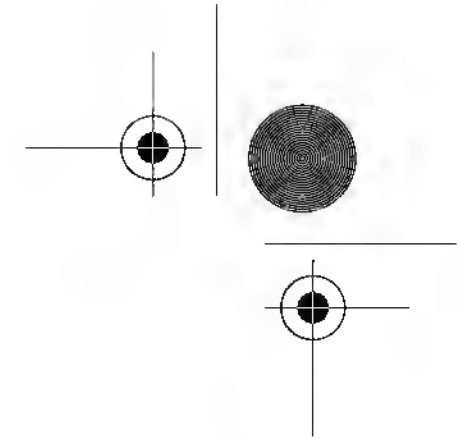
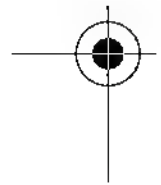
where T , E_a , and R have their usual meaning and T_{ref} is a reference temperature at which the shelf life is known.

RRS models are interesting because they enable shelf life to be predicted at different temperatures and for products where the specific spoilage organisms or the type of reaction responsible for spoilage are not known. For an unusually temperature-sensitive modified atmosphere packed shrimp product ($E_a > 100$ kJ/mol), Equation 3.40 described the effect of temperature (0 to 25°C) on shelf life more appropriately than a similarly formulated RRS model relying on the square-root model (Equation 3.1). However, a simple exponential RRS model was as useful as Equation 3.40. That the Arrhenius and exponential RRS models performed better than the square-root model was due to the fact that different groups of microorganisms were responsible for spoilage at low and high storage temperatures, respectively (Dalgaard and Jørgensen, 2000). This situation is common and a reason why entirely empirical RRS models can be more appropriate for shelf-life prediction than kinetic models relying on growth of known spoilage microorganisms. In fact, kinetic models for growth of spoilage bacteria are generally useful only for shelf-life prediction within the spoilage domain of a specific microorganism (Dalgaard, 2002).

3.2.5 POLYNOMIAL AND CONSTRAINED LINEAR POLYNOMIAL MODELS

Of the types of secondary models applied within predictive microbiology polynomial models are probably the most common. As shown in Table 3.5 and Table 3.6,





the effect of many different environmental parameters (e.g., temperature, NaCl/ a_w , pH, nitrite, CO₂, organic acids, and natural antimicrobials) has been described by these linear models. Polynomial models were extensively used during the 1990s and they remain widely applied although square-root-type and CPMs are becoming increasingly popular (Table 3.5). Polynomial models are attractive, first, because they are relatively easy to fit to experimental data by multiple linear regression, which is available in most statistical packages. Second, polynomial models allow virtually any of the environmental parameters and their interactions to be taken into account. Thus, application of polynomial models is a simple way to summarize information from a data set. Once the coefficients in a polynomial model have been estimated, the information is easy to use particularly if the model is included in application software. In fact, the application software packages Pathogen Modeling Program and Food MicroModel rely primarily on the use of polynomial models (www.arserrc.gov/mfs/pathogen.htm; Buchanan, 1993a; McClure et al., 1994a).

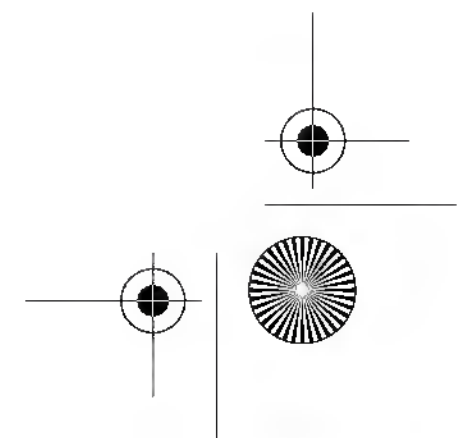
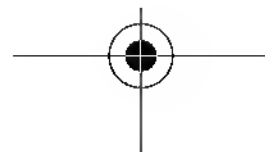
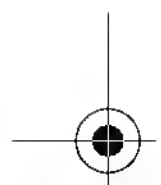
To illustrate the use of polynomial models a quadratic equation used by McClure et al. (1993) is shown below (Equation 3.41):

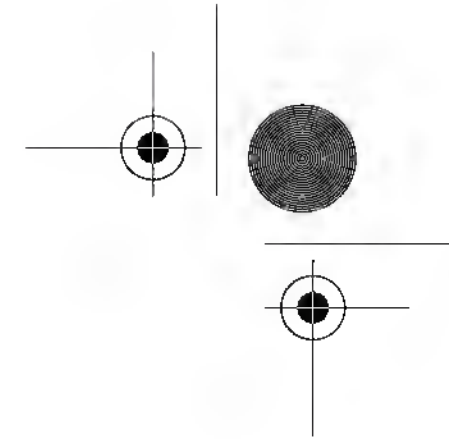
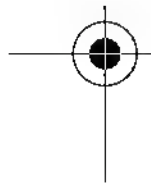
$$\begin{aligned} \ln y = & p_1 + p_2x_1 + p_3x_2 + p_4x_3 + p_5x_1x_2 + p_6x_1x_3 \\ & + p_7x_2x_3 + p_8x_1^2 + p_9x_2^2 + p_{10}x_3^2 + e \end{aligned} \quad (3.41)$$

where $\ln y$ denotes the natural logarithm of the modeled growth responses ($y = \mu_{\max}$, lag time or maximum population density [MPD], or the modified Gompertz model parameters B or M); p_i ($i = 1, \dots, 10$) are the coefficients to be estimated; x_1 is the temperature (°C); x_2 is the pH; x_3 is NaCl (% w/v); e is a random error supposed to have zero mean and constant variance.

As shown by Equation 3.41 the same polynomial equation can be used to model different microbial growth responses. Actually, many studies have modeled the effect of environmental conditions on specific parameters in primary growth models, particularly B , M , and C in the modified Gompertz model. Measures of lag time, growth rate, or time for, e.g., a 1000-fold increase in the cell concentration are then calculated at specific environmental conditions from the predicted value of B , M , and C (Buchanan and Phillips, 2000; Eifert et al., 1997; Skinner et al., 1994; Zaika et al., 1998). Growth responses to be modeled are typically ln- or log₁₀-transformed (Equation 3.41) and it is common practice to transform the growth response without transforming the model.

However, polynomial models have properties that limit their usefulness as secondary predictive models. Polynomials include many coefficients that have no biological interpretation. As an example, Equation 3.41 uses 10 coefficients to model the effect of three environmental parameters. With four environmental parameters, polynomials with 15 coefficients are frequently used. The high number of coefficients and their lack of biological interpretation make it difficult to compare polynomial models with other secondary predictive models. The important information included in, e.g., the T_{\min} parameter of a square-root-type model, is not provided by a polynomial model.



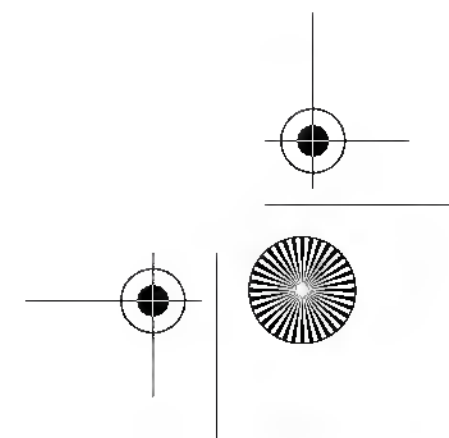
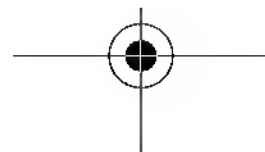
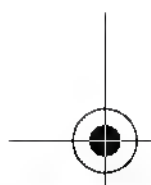
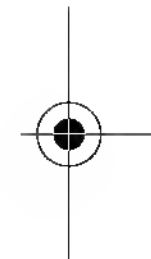
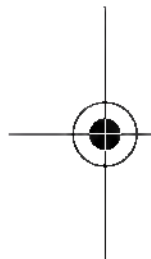


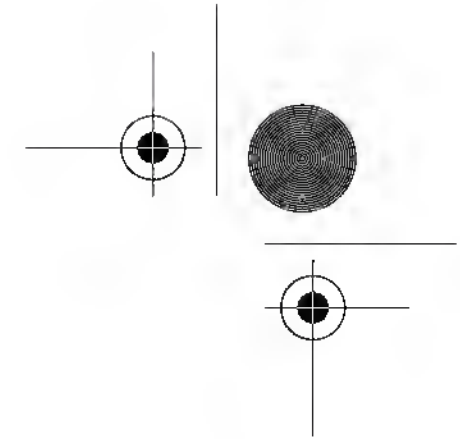
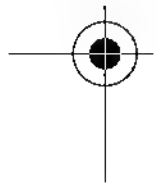
Higher order polynomial models, e.g., cubic or quadratic models have been criticized for being too flexible and for attempting to model, rather than eliminate, experimental error (Chapter 4; Baranyi et al., 1996; Sutherland et al., 1996). Because of the very flexible nature of higher order polynomial models they should not be used as secondary models within predictive microbiology unless very high quality experimental data are available and support the application of these models. Furthermore, because quadratic polynomial models are highly flexible they should only be used to provide predictions by interpolation. Baranyi et al. (1996) pointed out that the interpolation region of a polynomial model is the minimum convex polyhedron (MCP) defined by the ranges of the environmental parameters used to develop the model, i.e., the experimental design. These authors also stressed that the interpolation region (Figure 3.10) can be substantially smaller than the rectangular parallelepiped whose sides are given by the endpoints of the ranges of environmental parameters, termed the “nominal variable space” (Baranyi et al., 1996).

Determination of the interpolation region of a polynomial model is not self-evident and requires information about ranges of the environmental parameters used to develop the model. Pin et al. (2000) suggested a method to determine if a specific environmental condition is inside or outside the interpolation region of a particular polynomial model. This method relies on the iterative algorithm used by the Solver add-in of Microsoft Excel and thus is readily accessible to many users. However, we believe for it to become widely used the calculation of interpolation regions should be included in dedicated predictive modeling application software.

To overcome the problem that quadratic polynomial models can be too flexible, and therefore in some situations provide predictions that are not logical, the application of constrained polynomial models was recently suggested (Geeraerd et al., in press). With this approach, the basic idea is to combine *a priori* information about the effect of environmental parameters on growth responses with classical polynomial models. For example, at suboptimal conditions it was assumed that the growth rate should always increase for increasing temperature and a_w values and decrease for increasing CO_2 levels. Thus, the partial derivative of the model with respect to temperature and a_w should always be positive whereas the partial derivative of the model with respect to CO_2 should always be negative. Coefficients of the polynomial model were then fitted with the constraints obeyed at all edges of the experimental design. The constrained polynomial model was fitted by the usual process of minimizing the sum of squared errors and the fitting was carried out using the Optimization Toolbox within the MatLab software (Geeraerd et al., in press). As compared to classical polynomial models, constrained polynomial models have the clear advantage of being more robust but the clear disadvantage of being substantially more difficult to fit. Simplification of the fitting process seems necessary before constrained polynomial models find wide application in predictive microbiology.

Masana and Baranyi (2000a) described methods for integration of new data into existing polynomial models, pointing out that the interpolation region of the newly developed model can be unexpectedly small and also presenting methods for quantifying the increased risk of inadvertent extrapolation (Baranyi et al., 1996). Polynomial models feature many cross-product terms, making the addition of new terms much more complex than with models embodying the gamma concept (Section





3.2.2). Nonetheless, when expanding a model by the addition of data for a new variable, Masana and Baranyi (2000b) demonstrated that the original model can be retained as a special case of the expanded model, by holding the terms of the original model, i.e., those that do not contain the new variable, as constants during the modeling process for the expanded model.

3.2.6 ARTIFICIAL NEURAL NETWORKS

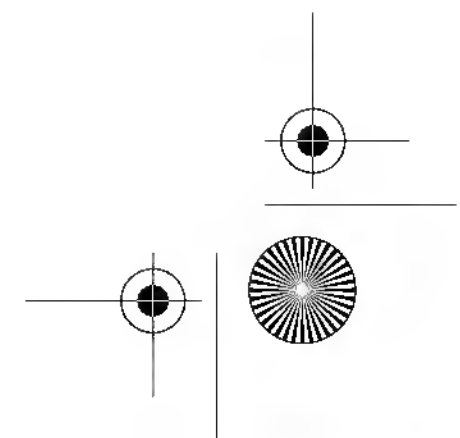
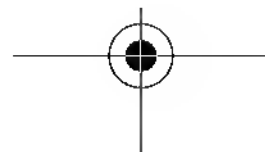
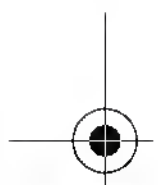
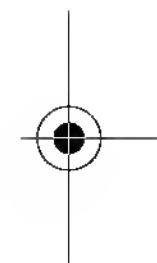
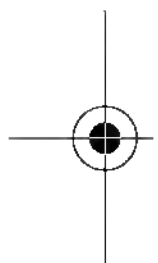
Artificial neural networks (ANNs) are algorithms that can be used to perform complex statistical modeling between a set of predictor variables and response variables. Their particular advantage is that they have the potential to approximate underlying relationships of any complexity between those variables. They have been used to generate secondary models for microbial growth rates and lag times (Garcia-Gimeno et al., 2002, 2003; Geeraerd et al., 1998a; Jeyamkondan et al., 2001; Lou and Nakai, 2001; Najjar et al., 1997), growth under fluctuating environmental conditions (Cheroutre-Vialette and Lebert, 2000; Geeraerd et al., 1998a), microbial inactivation (Geeraerd et al., 1998b), and have been proposed as an alternative to logistic regression modeling techniques (Tu, 1996). Their potential to replace logistic regression for growth limits modeling (see Section 3.4) has also been described (Hajmeer and Basheer, 2002, 2003a,b) in which context they have been termed “probabilistic neural networks” (PNNs).

Hajmeer et al. (1997) and Hajmeer and Basheer (2002, 2003a) describe the principles of ANNs and related technologies in the context of predictive microbiology, and numerous texts are dedicated to the subject but the following is largely drawn from the succinct and lucid description of Tu (1996).

Artificial neural networks were conceived decades ago by researchers attempting to reproduce the function of the human brain, i.e., its ability to learn and remember, but it was only in the 1980s that the “back-propagation” technique was rediscovered, enabling such computational systems to “learn” mathematical relationships between input and output variables.

Neural networks are effectively a series of mathematical relationships between predictor variables (“input nodes”), a series of hidden “nodes,” and an output variable (“output node”) (Figure 3.6). Each input node is related to each hidden node, and each hidden node is related to the output node, by some mathematical function. Each input is given a weight during the “training” routines, the value of each hidden node being the sum of a weighted linear combination of the input node values. In addition, bias values can be added to the weighted values of the inputs. These are analogous to the intercept in regression equations, while the weights are analogous to coefficients of the independent variables. The output node receives a weighted input from each of the hidden nodes, to which is often applied a logistic transformation or other function (the “activation function”) to determine the overall output.

A set of input and corresponding output values is presented to the network, the error is evaluated, and the weights are then adjusted to minimize the difference between the predicted output and that which was observed. This process of adjustment of weights is the back-propagation step and involves algorithms based on complex equations. Input data are continuously presented to the neural network until



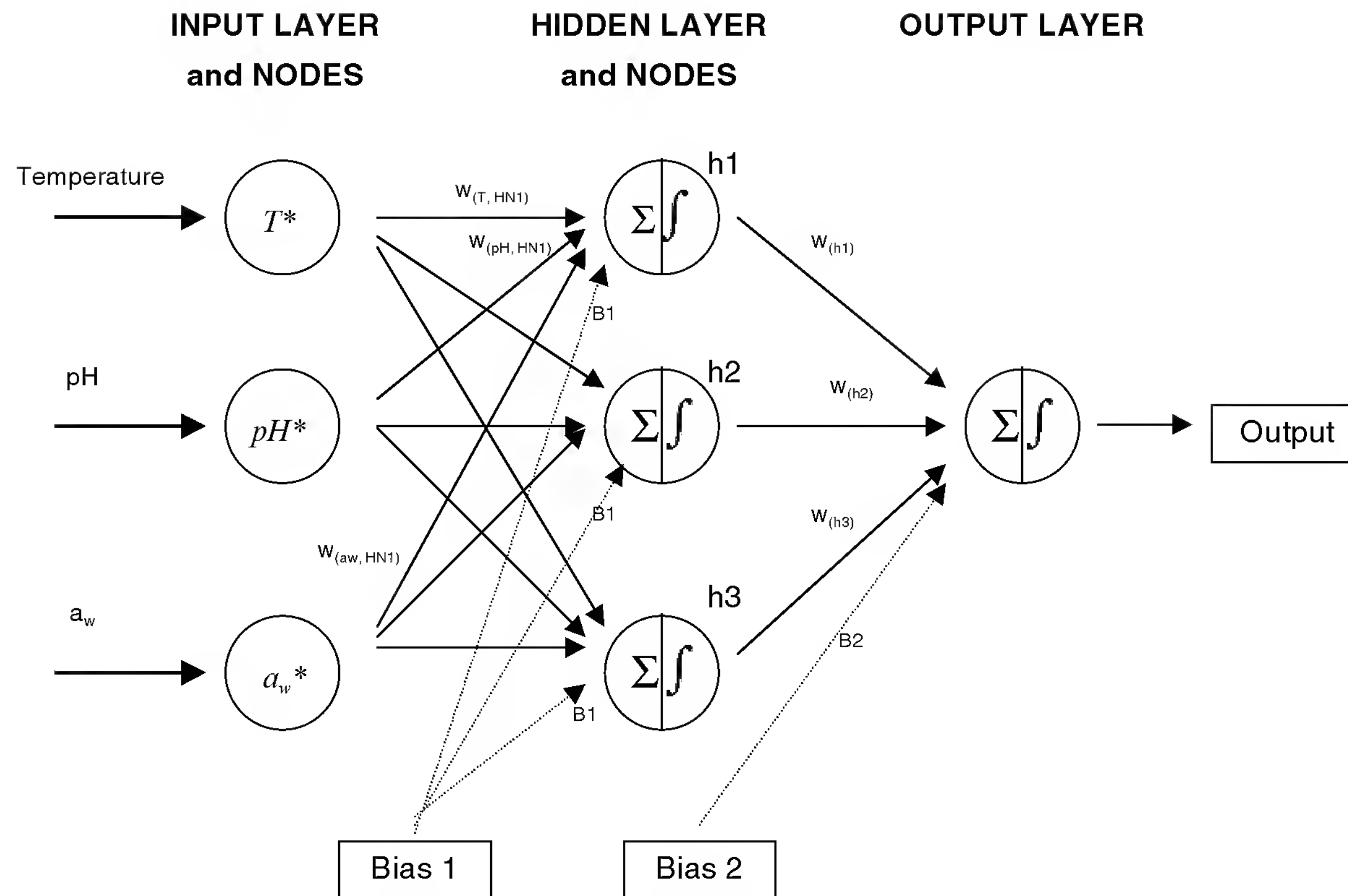
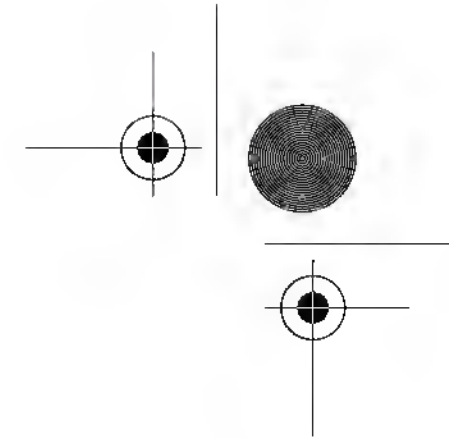
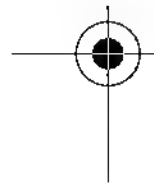


FIGURE 3.6 Diagram of an imaginary artificial neural network that might be used in predictive microbiology. The output is the response of the population of microorganisms to variations in the temperature, pH, and water activity of their growth medium. (The diagram is fully explained in the text.)



the overall error has been minimized, a process analogous to the iterative routines employed in nonlinear regression software. Optimal training algorithms can, at this time, only be determined empirically. Additionally, when using ANNs other elements of the modeling require experimentation, including the number of training cycles (too many can reduce predictive performance), the number of nodes in the hidden layer, and the ideal learning rate (the magnitude of change in the weights for each training case).

In Figure 3.6, the input, hidden, and output layers are shown, as well as the connections between them. Nodes are represented by circles. The $W_{(i,j)}$ terms indicate the weight applied to the inputs to hidden nodes. (Not all weights are represented in the diagram.) The hidden nodes have a transformation applied to them, e.g., a logistic function represented by the functions $h1$, $h2$, etc. Thus, in the example:

$$h1 = 1/(1+\exp(\text{Bias } 1 + W_{(T, \text{HN1})} \times T^* + W_{(\text{pH}, \text{HN1})} \times \text{pH}^* + W_{(\text{aw}, \text{HN1})} \times a_w^*))$$

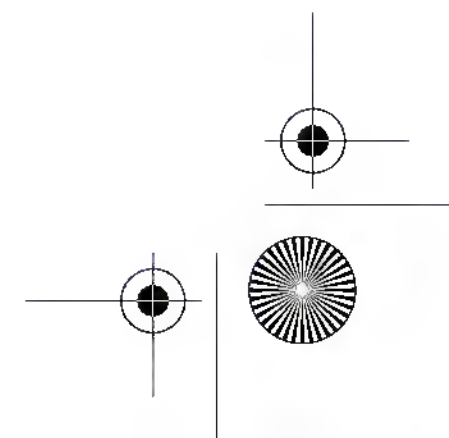
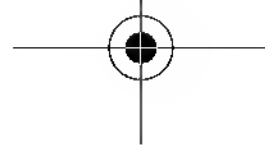
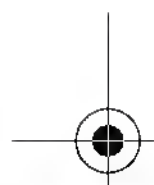
and, similarly:

$$\text{Output} = 1/(1 + \exp(\text{B2} + W_{(h1)} + W_{(h2)}))$$

Sufficient data are required so that a subset of data can be used to train the ANN, while the remainder is used to test the predictive ability of the ANN. One complete cycle of the training data set is called an “epoch” and the duration of the training is often described as the number of epochs required to minimize the error in the training set.

Tu (1996) compared the advantages and disadvantages of the ANN approach to those of traditional statistical regression modeling, as summarized in Table 3.4. Evaluation of the approach as applied to predictive models for microbial growth is presented below, and in relation to growth limits models in Section 3.4.2. Further comment is provided in Chapter 4, Section 4.4.3.

The use of ANN in predictive growth modeling remains relatively little developed, and direct comparison of the performance of different ANN techniques is still lacking. To describe growth curves, Schepers et al. (2000) concluded ANN was less appropriate than classical nonlinear sigmoidal growth models. Cheroutre-Vialette and Lebert (2000), however, found a recurrent (i.e., back-propagation) ANN suitable to predict growth of *Listeria monocytogenes* under constant and fluctuating pH and NaCl conditions. As shown in Table 3.5 and Table 3.6, several secondary ANN models have been developed including models for *Aeromonas hydrophila*, *Brochothrix thermosphaca*, *Escherichia coli*, lactic acid bacteria, *Listeria monocytogenes*, and *Shigella exneri*. These secondary ANN models have been compared with polynomial, square-root-type, and cardinal parameter models. The comparisons showed ANN typically fitted experimental data better and in most cases provided slightly more accurate predictions. Thus, in general, ANN may provide slightly improved predictions. Commercial neural network software is available and development of ANNs has become relatively easy. However, ANN is a data-driven approach and this could be a drawback because a secondary model that can be written as an equation with coefficients and parameters is not produced. The



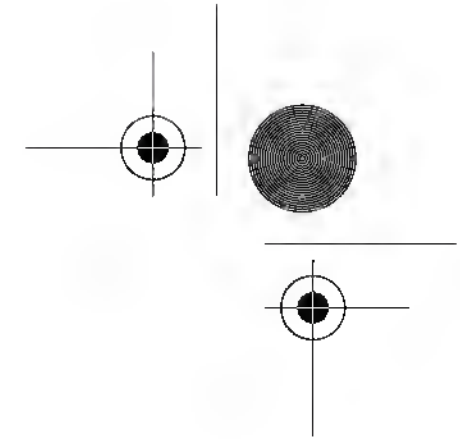
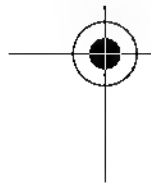


TABLE 3.4
Advantages and Disadvantages of Neural Network Approaches to Modeling

| Disadvantages | Advantages |
|--|--|
| Neural networks are a “black box” and have limited ability to specifically identify possible causal relationships between predictor and response variables | Neural networks require less formal statistical training to develop |
| Neural network models may be more difficult to use in the field | Neural network models can implicitly detect complex nonlinear relationships between predictor and response variables |
| Neural network modeling requires greater computational resources | Neural network models have the ability to detect all possible interactions between predictor variables |
| Neural network models are prone to overfitting | Neural networks can be developed using multiple different training algorithms Neural network model development is empirical, and many methodological issues remain to be resolved |

Source: After Tu, J.V. *J. Clin. Epidemiol.*, 11, 1225–1231, 1996.

incorporation of classical secondary models in user-friendly application software has been essential for the usefulness of predictive microbiology in industry, teaching, and research. It remains to be demonstrated whether successful ANN models can, in a similar way, be communicated to and conveniently applied by wide groups of users within predictive microbiology.

3.3 SECONDARY MODELS FOR INACTIVATION

There are relatively few models that consider the effects of multiple environmental factors on the rate of death of microorganisms, and these are discussed in [Chapter 2](#) and [Chapter 5](#). Some available inactivation models are also summarized in [Table 3.5](#) and [Table 3.6](#).

3.4 PROBABILITY MODELS

3.4.1 INTRODUCTION

Models to predict the likelihood, as a function of intrinsic and extrinsic factors, that growth of a microorganism of concern could occur in a food were first explored in the 1970s. Those models were concerned with prediction of the probability of formation of staphylococcal enterotoxin or botulinum toxin within a specified period of time under defined conditions of storage and product composition (Genigeorgis, 1981; Gibson et al., 1987). Phenomena that have been modeled using this approach

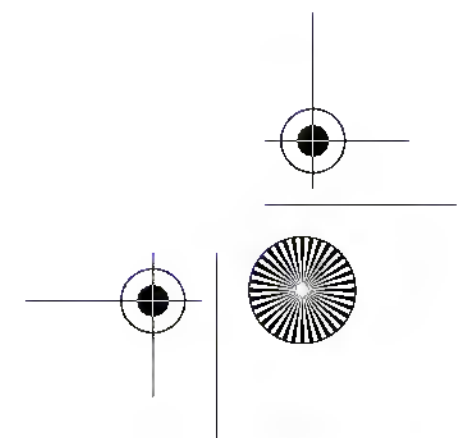
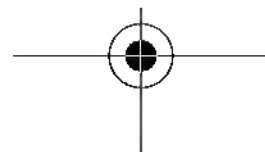
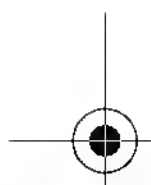


TABLE 3.5
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

| Microorganisms and References | Type of Secondary Model | Response Variables | Independent Variables and Ranges |
|---|----------------------------|------------------------------|--|
| <i>Aeromonas hydrophila</i> | | | |
| Palumbo et al. (1992) ^a | Polynomial | GT, ^b lag | <i>T</i> (5–42°C); NaCl (0.5–4.5%); pH (5.0–7.3); Na-nitrite (0–200 ppm); anaerobic |
| McClure et al. (1994b) | Polynomial | GT, lag | <i>T</i> (3–20°C); NaCl (0.5–4.5%); pH (4.6–7.0); aerobic |
| Palumbo et al. (1996) ^a | Polynomial | GT, lag | <i>T</i> (5–42°C); NaCl (0.5–4.5%); pH (5.0–7.3); Na-nitrite (0–200 ppm); aerobic |
| Devlieghere et al. (2000a) | Square-root, polynomial | μ_{\max} , lag | <i>T</i> (4–12°C); <i>a_w</i> (0.974–0.992); CO ₂ (0–2403 ppm); pH 6.12; nitrite (22 ppm) |
| Jeyamkondan et al. (2001) | ANN | GT, lag | Data from McClure et al (1994b) |
| <i>Aspergillus</i> spp. | | | |
| Pitt (1995) | Kinetic with yield factors | Growth and a toxin formation | <i>T</i> ; <i>a_w</i> ; pH; and colony size: limits not specified in manuscript |
| Molina and Giannuzzi (1999) | Arrhenius | Colony growth | <i>T</i> (25–36°C); propionic acid (129–516 ppm) |
| Rosso and Robinson (2001) | CPM | Colony growth | <i>T</i> (25, 30, 37°C); <i>a_w</i> (0.83–0.99); pH (6.5); humectant: glucose/fructose |
| Sautour et al. (2001) | CPM | Colony growth | <i>T</i> (25°C); <i>a_w</i> (0.88–0.99) |
| <i>Bacillus cereus</i> | | | |
| Benedict et al. (1993) ^a | Polynomial | GT, lag | <i>T</i> (5–42°C); NaCl (0.5–5.0%); pH (4.5–7.5); Na-nitrite (0–200 ppm); aerobic |
| Sutherland et al. (1996) ^c | Polynomial | GT, lag | <i>T</i> (10–30°C); NaCl (0.5–10.5%); pH (4.5–7.0); CO ₂ (10–80%) |
| Zwietering et al. (1996) | Gamma | μ_{\max} | <i>T</i> (10–30°C); <i>a_w</i> (0.95–1.00); pH (4.9–6.6) |
| Chorin et al. (1997) | Polynomial | Growth rate, lag | <i>T</i> (7–30°C); <i>a_w</i> (0.95–0.991); pH (5–7.5); humectant: glycerol |
| Singaglia et al. (2002) | Polynomial | Spore germination | <i>T</i> (20–40°C); <i>a_w</i> (0.94–0.99); pH (4.5–6.5) |
| <i>Clostridium botulinum</i> | | | |
| Baker and Genigeorgis (1990) ^a | Polynomial | Time to toxin formation | <i>T</i> (4–30°C); initial spore conc. (–2 to +4 log cfu/g); initial aerobic plate count (–2 to +3 log cfu/g) |
| Graham et al. (1996) ^c | Polynomial | GT, time to toxin | <i>T</i> (4–30°C); NaCl (1.0–5.0%); pH (5.0–7.3) |

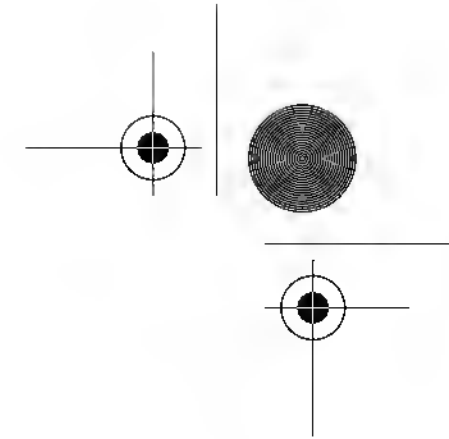
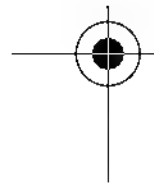
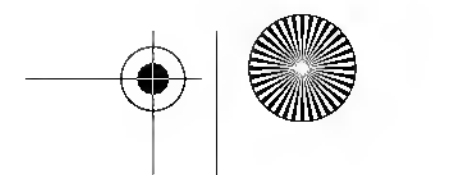
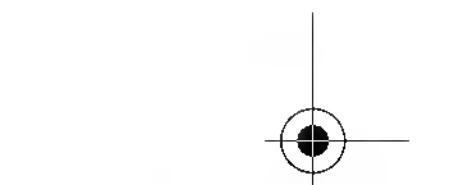
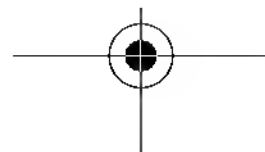
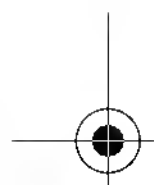


TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

| Microorganisms and References | Type of Secondary Model | Response Variables | Independent Variables and Ranges |
|---|-------------------------|---------------------------------------|---|
| Whiting and Oriente (1997) ^a | Polynomial | Time to turbidity | T (4–28°C); NaCl (0–4%); pH (5–7); initial spore conc. (1–5 log cfu/g) |
| Chea et al. (2000) | Polynomial | Spore germination | T (15–30°C); NaCl (0.5–4.0%); pH (5.5–6.5) |
| Fernandez et al. (2001) | Polynomial | Time to turbidity | T (5–12°C); NaCl (0.5–2.5%); pH (5.5, 6.5); CO ₂ (0–90%) |
| <i>Clostridium perfringens</i> | | | |
| Juneja et al. (1996) ^a | Polynomial | GT, lag | T (12–42°C); NaCl (0–3%); pH (5.5–7.0); Na-pyrophosphate (0–3%) |
| <i>Escherichia coli</i> | | | |
| Buchanan and Bagi (1994) ^a | Polynomial | GT, lag | T (5–42°C); NaCl (0.5–5.0%); pH (4.5–8.5); Na-nitrite (0–200 ppm); aerobic and anaerobic |
| Sutherland et al. (1995, 1997) ^c | Polynomial | GT, lag | T (10–30°C); NaCl (0.5–6.5%); pH (4.0–7.0); Na-nitrite (0–200 ppm); aerobic |
| Rasch (2002) | Polynomial | Growth rate | T (10–30°C); NaCl (0.5–3.0%); pH (4.5–6.5); reuterin (0–4 AU/ml) |
| Ross et al. (2003) | Square-root | GT | T (7.6–47.4°C); a_w (0.951–0.999); pH (4.02–8.28); lactic acid (0–500 mM) |
| Skandamins et al. (2002) | Vitalistic | Time to decline in cell concentration | T (0–15°C); pH (4.0–5.0); oregano essential oil (0.0–2.1%) |
| Garcia-Gimeno et al. (2003) | ANN | Growth rate, lag time | T (9–21°C); NaCl (0–8%); pH (4.5–8.5); Na-nitrite (0–200 ppm) |
| Whiting and Golden (2003) | Polynomial | Time to decline in cell concentration | T (4–37°C); NaCl (0–15%); pH (3.5–7.0); Na-lactate (0–2%); Na-nitrite (0–75 ppm) |
| <i>Listeria monocytogenes</i> ^d | | | |
| Buchanan et al. (1997) | Polynomial | Time to decline in cell concentration | T (4–42°C); NaCl (0.5–19%); pH (3.2–7.3); lactic acid (0–2%); Na-nitrite (0–200 ppm) |
| Razavilar and Genigeorgis (1998) | Polynomial | Probability of growth | T (4–30°C); NaCl (0.5–12.5%); methylparaben (0–0.2%); pH (~5.9); K-sorbate (0.3%); Na-propionate (0.1%); Na-benzoate (0.1%) |
| Cheroutre-Vialette and Lebert (2000) | ANN | Absorbance at 600 nm | T (20°C); pH (5.6–9.5); NaCl (0–8%) |



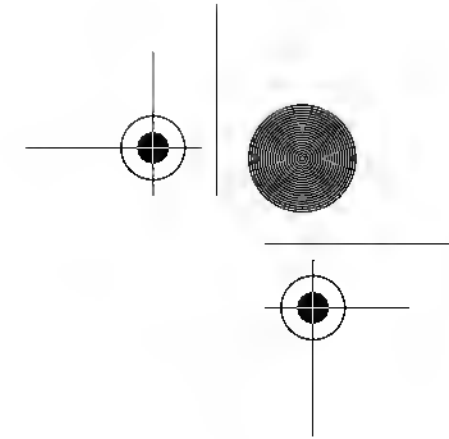
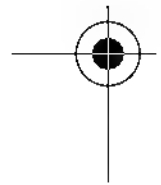
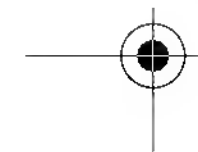
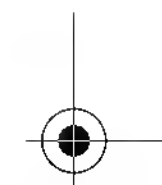
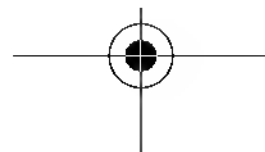
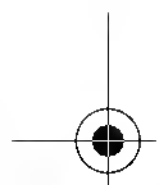
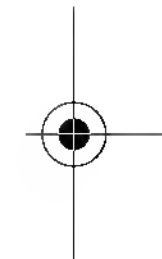
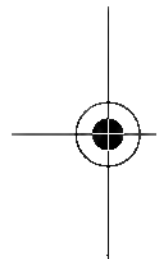


TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

| Microorganisms and References | Type of Secondary Model | Response Variables | Independent Variables and Ranges |
|--------------------------------|-------------------------|--------------------|--|
| Bouttefroy et al. (2000) | Polynomial | Cell concentration | T (22°C); pH (5.0–8.2); NaCl (0–6%); curvaticin 13 (0–160 AU/ml) |
| Rodriguez et al. (2000) | Arrhenius | μ_{\max} | T (4–20°C) |
| Augustin and Carlier (2000a,b) | CPM | μ_{\max} , lag | T (–2.7 to –45.5°C); a_w (0.910–0.997); pH (4.55–9.61); acetic acid (0–20.1 mM); lactic acid (0–5.4 mM); citric acid (0–1.6 mM); Na-benzoate (0–0.7 mM); K-sorbate (0–5.1 mM); Na-nitrite (0–11.4 μ M); glycerol monolaurin (0–118.5 ppm); butylated hydroxyanisole (0–254 ppm); butylated hydroxytoluene (0–48.7 ppm); <i>tert</i> -butylhydroquinone (0–1400 ppm); CO ₂ (0–1.64 proportion); caffeine (0–10.8 g/l); phenol (0–12.5 ppm) |
| Ross et al. (in press) | Square-root | μ_{\max} | T (3–40 °C); a_w (0.920–0.997); pH (4.0–7.8); lactic acid (0–450 mM); nitrite (0–150 ppm) |
| Buchanan and Phillips (2000) | Polynomial | GT, lag | T (4–37°C); pH (4.5–7.5); NaCl (0.5–10.5%); Na-nitrite (0–1000 ppm); aerobic |
| Buchanan and Phillips (2000) | Polynomial | GT, lag | T (4–37°C); pH (4.5–8.0); NaCl (0.5–5.0%); Na-nitrite (0–1000 ppm); anaerobic |
| Devlieghere et al. (2001) | Square-root, polynomial | μ_{\max} , lag | T (4–12°C); a_w (0.9622–0.9883); Na-lactate (0–3.0%); Na-nitrite (20 ppm); pH (6.2) |
| Le Marc et al. (2002) | CPM | μ_{\max} , lag | T (0.5–43°C); pH (4.5–9.4); acetic acid (16–64 mM); lactic acid (40–138 mM); propionic acid (18–55 mM) |
| Seman et al. (2002) | Polynomial | Growth rate | T (4°C); NaCl (0.8–3.6%); Na-diacetate (0.0–0.2%); K-lactate (0.15–5.6%); Na-erythroate (317 ppm); Na-nitrite (97 ppm); Na-tripolyphosphate (0.276%) |



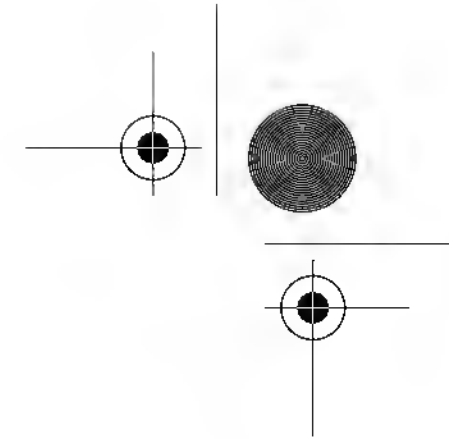
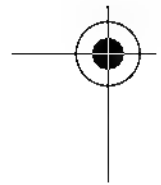
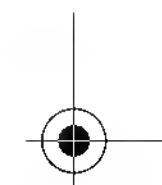
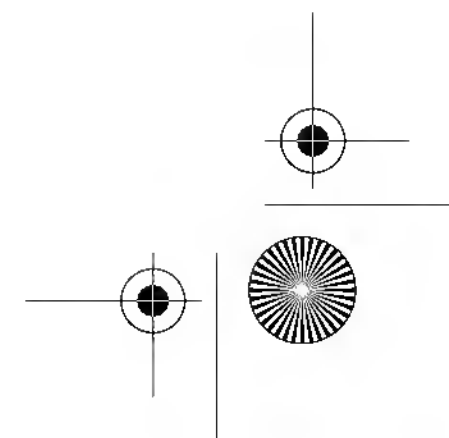
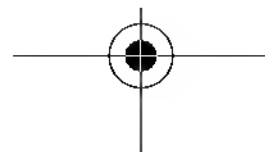
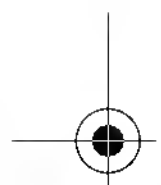
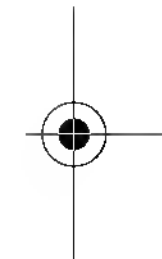
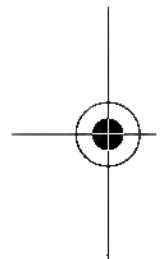


TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

| Microorganisms and References | Type of Secondary Model | Response Variables | Independent Variables and Ranges |
|--|-------------------------|---------------------------------------|--|
| Gimenez and Dalgaard (in press) | Square-root | μ_{\max} | T (4–10°C); %WPS (2–6%); smoke components/phenol (3–10 ppm); pH (5.9–6.3); lactic acid (0–20,000 ppm); interaction with lactic acid bacteria |
| <i>Salmonella</i> | | | |
| Gibson et al. (1988) ^c | Polynomial | GT, lag | T (10–30°C); NaCl (0.5–4.5%); pH (5.6–6.8); aerobic |
| Davey and Daughtry (1995) | Arrhenius | Growth rate, lag | Data from Gibson et al. (1988) |
| Koutsoumanis et al. (1998) | Polynomial | μ_{\max} | T (22–42°C); pH (5.5–7.0); oleuropein (0–0.8%); aerobic |
| Oscar (1999) | Polynomial | μ_{\max} , lag | T (15–40°C); pH (5.2–7.4); previous pH (5.7–8.6); aerobic |
| Oscar (2002) | Square-root, CPM | μ_{\max} , lag | T (8–48°C); aerobic |
| Skandamins et al. (2002) | Vitalistic | Time to decline in cell concentration | T (5–20°C); pH (4.3–5.3); oregano essential oil (0.5–2.0%) |
| <i>Shigella</i> | | | |
| Zaika et al. (1994, 1998) ^a | Polynomial | GT, lag | T (10–37°C); NaCl (0.5–5.0%); pH (5.0–7.5); Na-nitrite (0–1000 ppm); aerobic |
| Jeyamkondan et al. (2001) | ANN | GT, lag | Data from Zaika et al. (1994) |
| <i>Staphylococcus aureus</i> | | | |
| Ross and McMeekin (1991) | Square-root | Growth rate | T (5–35°C); a_w (0.848–0.997) |
| Buchanan et al. (1993) ^a | Polynomial | GT, lag | T (12–45°C); NaCl (0.5–16.5%); pH (4.5–9.0); Na-nitrite (1–200 ppm); aerobic and anaerobic |
| Dengremont and Membré (1997) | Square-root | μ_{\max} | T (10–37°C); NaCl (0–10%); pH (5–8) |
| Eifert et al. (1997) | Polynomial | Parameters in primary model | T (12–28°C); NaCl (0.5–8.5%); pH (5.0–7.0); acidulants HCl, acetic acid, or lactic acid; aerobic |
| <i>Vibrio</i> spp. | | | |
| Miles et al. (1997) | Square-root | GT | T (8–45°C); a_w (0.936–0.995); aerobic |



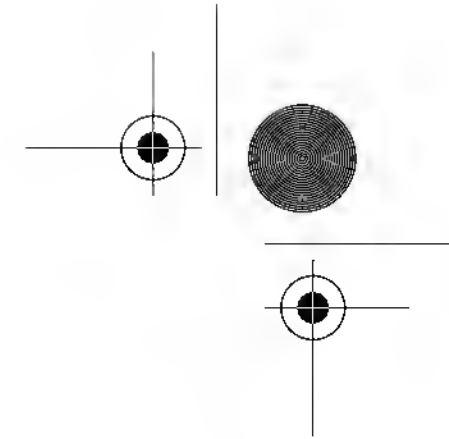
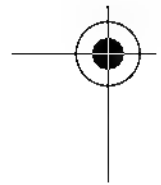


TABLE 3.5 (Continued)
Examples of Secondary Models for Growth of Pathogenic and Indicator Microorganisms

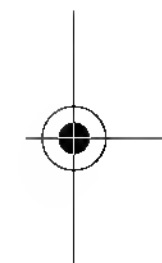
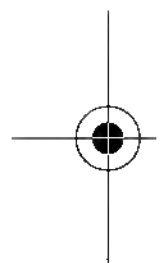
| Microorganisms and References | Type of Secondary Model | Response Variables | Independent Variables and Ranges |
|--|-------------------------|--------------------|---|
| <i>Yersinia</i> spp. | | | |
| Bhaduri et al. (1995) ^a | Polynomial | GT, lag | <i>T</i> (5–42°C); NaCl (0.5–5.0%); pH (4.5–8.5); Na-nitrite (0–200 ppm); aerobic |
| Sutherland and Bayliss (1994) ^c | Polynomial | GT, lag | <i>T</i> (5–30°C); NaCl (0.5–6.5%); pH (4.0–7.0); aerobic |
| Pin et al. (2000) | Polynomial | μ_{\max} , lag | <i>T</i> (1–8°C); CO ₂ (0–83%); O ₂ (0–60%) |
| Wei et al. (2001) | Square-root | μ_{\max} , lag | <i>T</i> (4–34°C); air; vacuum; CO ₂ 100% |

^a Models included in the Pathogen Modeling Program, which is available free of charge at www.arserrc.gov/mfs/PMP6_download.htm.

^b Generation time = $\ln(2)/\mu_{\max}$.

^c Model included in Food MicroModel. The values of model parameter are not included in the manuscript.

^d See Ross et al. (2000) for a list of *Listeria monocytogenes* growth models published prior to 2000.



include germination of spores, population growth, survival, and toxin formation. These types of models became known as “probability” models.

In the latter part of the 1990s it seemed that the only way to manage the risk to consumers from certain pathogens was to ensure that the organism was never present in foods, or to ensure that it was not able to grow in foods that could become contaminated. The latter imperative led to the re-development of “growth/no-growth boundary,” or “interface” modeling.

This section is divided into three main parts. In the first, Section 3.4.2, “traditional” probability modeling is briefly discussed. Section 3.4.3 presents and discusses the newer growth/no-growth (G/NG) modeling approaches, while Section 3.4.4 considers methodological issues relevant to probability and G/NG modeling.

3.4.2 PROBABILITY MODELS

Several reviews of probability modeling were presented in the early 1990s (Baker, 1993; Baker and Genigeorgis, 1993; Dodds, 1993; Lund, 1993; Ross and McMeekin, 1994; Whiting, 1995) but, possibly because of the relative paucity of new publications in this field since then, there has been no more recent dedicated review. Whiting and Oriente (1997) and Zhao et al. (2001), however, provide succinct updates.

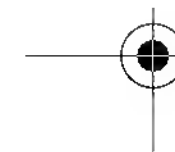
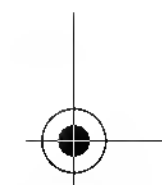
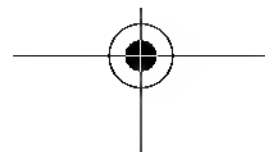
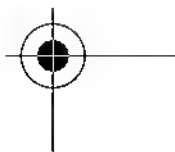
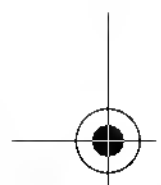


TABLE 3.6
Examples of Secondary Models for Growth of Spoilage Microorganisms

| Microorganisms and References | Type of Model | Response Variables | Independent Variables and Ranges |
|---|-------------------------|-------------------------------|--|
| <i>Bacillus</i> | | | |
| <i>stearothermophilus</i> Ng et al. (2002) | Polynomial | Growth rate, GOL ^a | <i>T</i> (45–60°C); NaCl (0–1.5%); pH (5.5–7.0) |
| <i>Brochothrix</i> | | | |
| <i>thermosphacta</i> | | | |
| McClure et al. (1993) | Polynomial | μ_{\max} , lag | <i>T</i> (1–30°C); NaCl (0.5–8.0%); pH (5.6–6.8); aerobic |
| Abdullah et al. (1994) | Polynomial | GT, lag, MPD | <i>T</i> (–2 to –10°C); CO ₂ (2–40%); diameter of meat particles (2–10 mm) |
| Geeraerd et al. (1998a) | ANN | μ_{\max} , lag | Data from McClure et al. (1993) |
| Pin and Baranyi (1998) | Polynomial | μ_{\max} , lag | <i>T</i> (2–11°C); pH (5.2–6.4); aerobic |
| Koutsoumanis et al. (2000) | Arrhenius | μ_{\max} | <i>T</i> (0–20°C); CO ₂ (0–100%) |
| Jeyamkondan et al. (2001) | ANN | GT, lag | Data from McClure et al. (1993) |
| <i>Chryseomonas</i> spp. | | | |
| Membré and Kubaczka (1998) | Square-root | μ_{\max} | <i>T</i> (1.3–10°C); aerobic |
| <i>Enterobacteriaceae</i> | | | |
| Pin and Baranyi (1998) | Polynomial | μ_{\max} , lag | <i>T</i> (2–11°C); pH (5.2–6.4); aerobic |
| Lactic acid bacteria | | | |
| Passos et al. (1993) | Kinetic | μ_{\max} | pH (3.8–6.0); lactic acid (0–30 mM); acetic acid (0–40 mM); NaCl (0–9%); cucumber juice |
| Gänzle et al. (1998) | Square-root | μ_{\max} | <i>T</i> (3–41°C); aerobic |
| Gänzle et al. (1998) | CPM | μ_{\max} | pH (4.2–6.7); ionic strength (0.0–1.97); acetate (0–0.2 mM); aerobic |
| Devlieghere et al. (2000a,b) | Square-root, polynomial | μ_{\max} , lag | <i>T</i> (4–12°C); <i>a_w</i> (0.962–0.9883); CO ₂ (0–1986 ppm); Na-lactate (0.0–3.0 5); pH 6.2 |
| Lou and Nakai (2001) | ANN | μ_{\max} , lag | <i>T</i> (4–12°C); <i>a_w</i> (0.962–0.9883); CO ₂ (0–2411 ppm); pH 6.2 Subset of data from Devlieghere et al. (2000a,b) |
| Wijtzes et al. (2001) | Square-root | μ_{\max} | <i>T</i> (3–30°C); <i>a_w</i> (0.932–0.990); pH (5.0–7.5) |

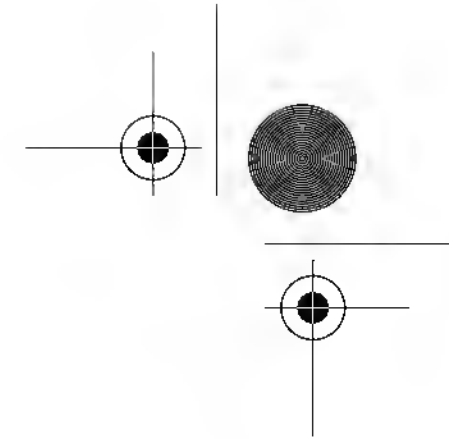
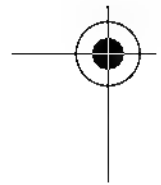


TABLE 3.6 (Continued)
Examples of Secondary Models for Growth of Spoilage Microorganisms

| Microorganisms and References | Type of Model | Response Variables | Independent Variables and Ranges |
|-------------------------------------|----------------------------|--------------------------------|--|
| Connil et al. (2002) | Polynomial | μ_{max} , lag | T (3–9°C); pH (2.5–6.5); glucose (0.2–0.6%) |
| Messens et al. (2002) | CPM | Growth, bacteriocin production | T (20–38°C); pH (4.8–7.0) |
| Leroy and De Vuyst (2003) | CPM | Growth, bacteriocin production | T (20–37°C); pH (4.5–6.5) |
| Garcia-Gimeno et al. (2002) | ANN | Growth rate, lag | T (20, 28°C); NaCl (0–6%); pH (4–7) |
| Messens et al. (2002) | CPM | Growth, bacteriocin production | T (20–38°C); pH (4.8–7.0) |
| Garcia-Gimeno et al. (2002) | ANN | Growth rate, lag | T (20, 28°C); NaCl (0–6%); pH (4–7) |
| Molds | | | |
| Gibson et al. (1994) | Polynomial | Colony growth | T (30°C); a_w (0.810–0.995) |
| Cuppers et al. (1997) | Square-root, CPM | Colony growth | T (5–37°C); NaCl (0–7%) |
| Valik et al. (1999) | Polynomial | Colony growth | T (25°C); a_w (0.87–0.995); aerobic |
| Batthey et al. (2001) | Polynomial | Probability of growth | T (25°C); pH (2.8–3.8); titratable acidity (0.2–0.6%); sugar content (8–16°Brix); Na-benzoate (100–350 ppm); K-sorbate (100–350 ppm) |
| Panagou et al. (2003) | Polynomial, Arrhenius, CPM | Colony growth | T (20–40°C); NaCl (2–10%); pH (3.5–5.0) |
| <i>Photobacterium phosphoreum</i> | | | |
| Dalgaard et al. (1997) ^b | Polynomial, square-root | μ_{max} | T (0–15°C); CO ₂ (0–100%) |
| <i>Pseudomonas</i> | | | |
| Membré and Burlot (1994) | Polynomial | μ_{max} , lag | T (4–30°C); pH (6–8); NaCl (0–5%) |
| Neumeyer et al. (1997) ^c | Square-root | GT | T (0–30°C); a_w (0.947–0.966) |
| Pin and Baranyi (1998) | Polynomial | μ_{max} , lag | T (2–11°C); pH (5.2–6.4); aerobic |

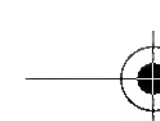
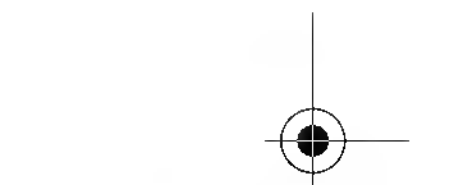
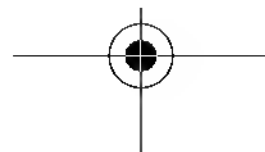
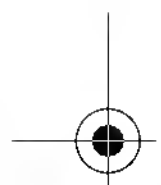
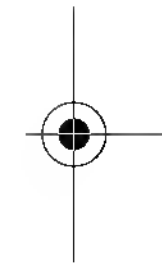
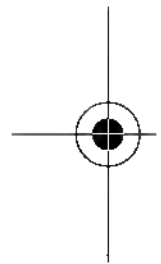


TABLE 3.6 (Continued)
Examples of Secondary Models for Growth of Spoilage Microorganisms

| Microorganisms and References | Type of Model | Response Variables | Independent Variables and Ranges |
|-------------------------------|---------------------------------|------------------------|--|
| Koutsoumanis et al. (2000) | Arrhenius | μ_{\max} | T (0–20°C); CO_2 (0–100%) |
| Koutsoumanis (2001) | Square-root | μ_{\max} , lag | T (0–15°C) |
| Rasmussen et al. (2002) | Process risk model | GT | Data from Neumeyer et al. (1997) |
| <i>Shewanella</i> spp. | | | |
| Dalgaard (1993) ^b | Square-root | μ_{\max} | T (0–35°C); aerobic, anaerobic |
| Koutsoumanis et al. (2000) | Arrhenius | μ_{\max} | T (0–20°C); CO_2 (0–100%) |
| Yeasts | | | |
| Deak and Beuchat (1994) | Polynomial | Changes in conductance | T (10–30°C); a_w (0.93–0.99); pH (3.8–4.6); K-sorbate (0–0.06%) |
| Passos et al. (1997) | Kinetic with product inhibition | μ_{\max} | T (30°C); pH (3.2–5.9); lactic acid (0–55 mM); acetic acid (0–35 mM); NaCl (0–6%); cucumber juice; aerobic and anaerobic |
| Gänzle et al. (1998) | Square-root | μ_{\max} | T (8–36°C); aerobic |
| Gänzle et al. (1998) | CPM | μ_{\max} | Ionic strength (0.0–3.2); acetic acid (0–90 mM); aerobic |

^a GOL = germination, outgrowth, and lag time.

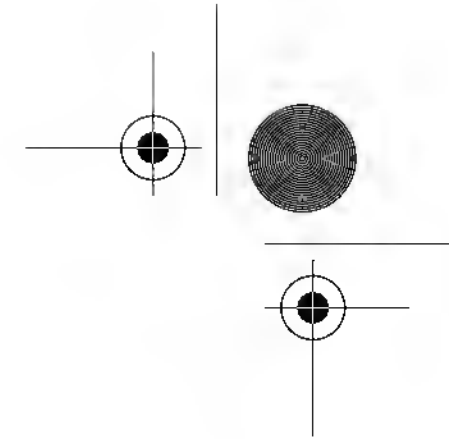
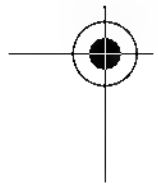
^b Model included in the Seafood Spoilage Predictor (SSP) software available free of charge at www.dfu.min.dk/micro/ssp/.

^c Model included in the Food Spoilage Predictor (FSP) software.

3.4.2.1 Logistic Regression

Dodds (1993) explains that in relation to the hazard presented by *Clostridium botulinum* in foods, the detection of the toxin is often more important than growth and that while growth is continuous and fairly easily determined, the presence of detectable toxin was seen as an “all-or-none” response. This led workers to seek methods to predict the probability of production of detectable toxin levels in response to the independent variables.

In probability models in predictive microbiology the data are usually that the response (e.g., growth, detectable toxin production) is observed under the experimental conditions, or that it is not. Responses such as detectable toxin production can be coded as either 0 (response not observed) or 1 (response observed) or, if repeated observations have been made, as probability (between 0 and 1). The probability is related to potential predictor variables by some mathematical function using regression techniques.



Logistic regression is a widely used statistical modeling technique — and is the technique of choice — when the outcome of interest is dichotomous (i.e., has only two possible outcomes). It is widely used in medical research (e.g., Hosmer and Lemeshow, 1989). Because regression techniques do not exist for dichotomous data, the regression equation is usually related to the log odds, or *logit*, of the outcome of interest. This has the effect of transforming the response variable from a binary response to one that extends from $-\infty$ to $+\infty$ reflecting the possible ranges of the predictor variables, and has desirable mathematical features also (Hosmer and Lemeshow, 1989). The logit function is defined as:

$$\text{logit } P = \log(P/(1 - P)) \quad (3.42)$$

where P is the probability of the outcome of interest. Logit P is commonly described as some function Y of the explanatory variables, i.e.:

$$\text{logit } P = Y \quad (3.43)$$

Equation 3.43 can be rearranged to:

$$1/(1 + e^{-Y}) = P$$

or

$$e^Y/(1 + e^Y) = P$$

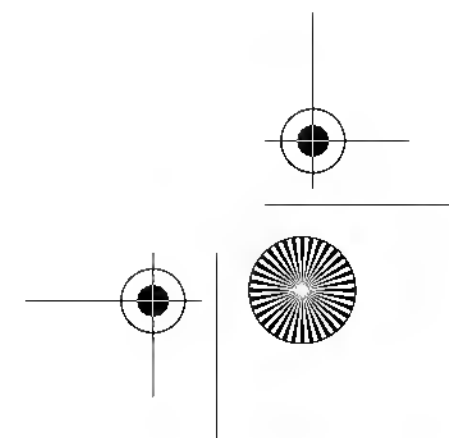
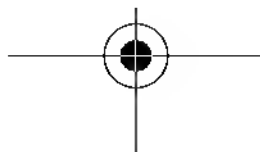
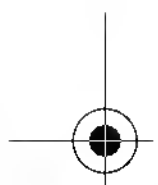
where Y is the function describing the effects of the independent variables.

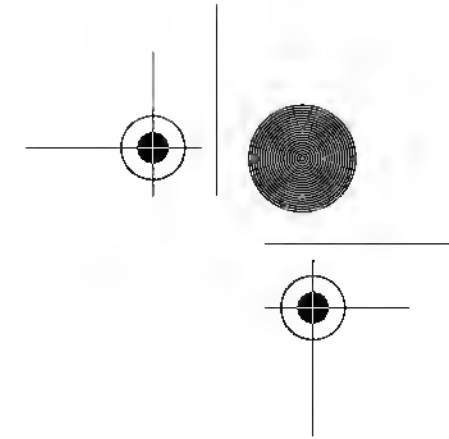
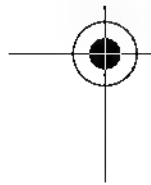
The latter parameterizations appear in some of the earlier probability modeling literature.

Zhao et al. (2001) assessed the performance of linear and logistic regression to model percentage data that are “bounded,” and may be considered as rescaled probability values. It was confirmed that logistic regression provided a much more accurate description of percentage data than linear regression, which had the insurmountable problem of predicting values outside the range of the data (i.e., less than 0% or greater than 100%).

3.4.2.2 Confounding Factors

Probability modelers used logistic regression to define the probability that detectable toxins would be produced within a specified period of time and under specified product composition and storage conditions. Models were based on the idea that a product was safe/acceptable or that it was not. Nonetheless, the responses measured in “probability modeling” were related to a number of factors that were in turn related to the *growth* of the organism under study and, in some cases, also included elements of survival. This approach appears to have arisen from the ideas of Riemann





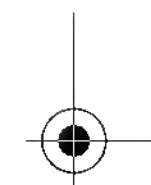
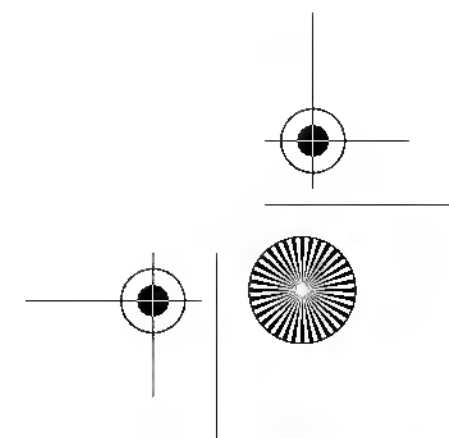
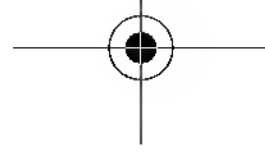
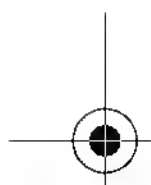
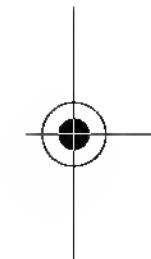
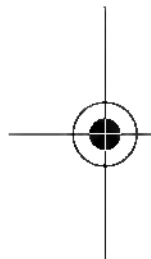
(1967) that the success of a preservation method with regard to *C. botulinum* is related to the probability that one spore will germinate and give rise to toxin in the finished product. In general, to assess the effect of preservation conditions on probability of toxin production, the probability of growth from a single cell is estimated as the number of spores able to initiate growth under the test conditions (usually determined by MPN [most probable number] methods) divided by the number originally inoculated (Lund, 1993). Often a series of increasingly dilute inocula are subjected to the test conditions to determine the minimum fraction able to initiate detectable growth under the test conditions.

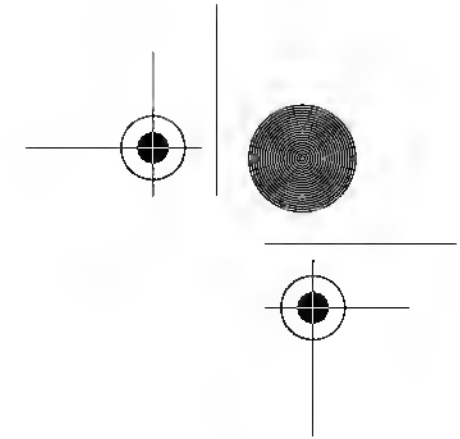
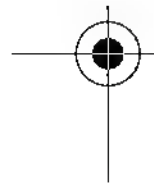
It might be expected that the probability of detection would increase with time. Indeed, Lindroth and Genigeorgis (1986) recognized that the probability of growth detection was also dependent upon the lag time of the inoculum, its initial density, and the duration of the study. They introduced a modification to the logit model to specifically model these effects. That model was subsequently used in a number of other studies (Baker et al., 1990; Ikawa and Genigeorgis, 1987). Whiting and Call (1993) criticized earlier models for probability of *C. botulinum* outgrowth and toxin production because they did not specifically monitor the time at which growth/toxin formation was first detected, and specifically modeled the probability of formation of toxin as a function of time and storage conditions using the logistic function, i.e., the probability of detectable growth, when plotted as a function of time, is a sigmoid curve. That approach was further refined (Whiting and Oriente, 1997; Whiting and Strobaugh, 1998) by inclusion of the inoculum density as an independent variable in the model.

Clearly, the probability of the responses in many of these traditional probability models is strongly related to the growth rate of the organism under the experimental conditions, leading Ross and McMeekin (1994) to conclude that the distinction that had traditionally been made between probability and kinetic models was an artificial one. Similarly, Baker et al. (1990) noted that “The rate of P increase ... expresses the growth rate ...”

However, under some experimental conditions P does not always reach an asymptote of 1. This is evident in the data of Whiting and colleagues (Whiting and Call, 1993; Whiting and Oriente, 1997; Whiting and Strobaugh, 1998), of Chea et al. (2000), and of Razavilar and Genigeorgis (1998). It had also been described earlier by Lund et al. (1987) who introduced to predictive microbiology a model that recognizes that under some conditions, no matter how long one waits, not all samples will show growth/toxigenesis.

While the above studies considered spores, Razavilar and Genigeorgis (1998) applied a logistic regression approach to the probability of growth initiation within 58 days of *Listeria monocytogenes* and other *Listeria* species in response to combinations of pH, salt, temperature, and methyl paraben, sodium propionate, sodium benzoate, and potassium sorbate (Table 3.5). Their results, also, suggested that under near-growth-limiting environmental conditions the asymptotic probability of growth (i.e., given in finite incubation time) was sometimes less than 1. Stewart et al. (2001) also commented that while kinetic models predict the mean growth rate, these estimates may be meaningless under stressed conditions owing to natural variability in biological responses. Similarly, Lund (1993) employed the





Gompertz model (see [Chapter 2](#)) to model the time-dependent probability of growth of *L. monocytogenes* Scott A as a function of environmental factors. Even at near-growth-limiting pH (4.3), however, the asymptote of the $\log(P_{\text{growth}})$ was still close to 1.

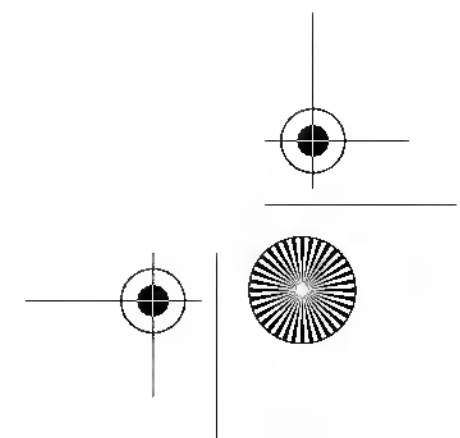
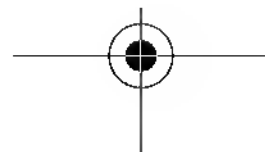
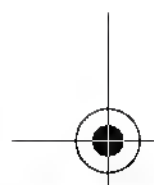
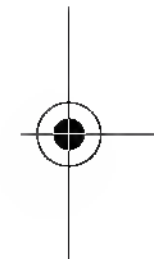
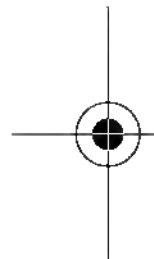
The above studies suggest that as environmental conditions become more inhibitory to growth, not only does the probability that growth will be observed during the course of the experiment decrease, but the probability that growth *is possible* also decreases. This may be because the generation or lag time of all cells within the inoculum becomes in nitely long. Under these conditions, one begins to identify the absolute limits to microbial growth under combined stresses, i.e., the G/NG interface.

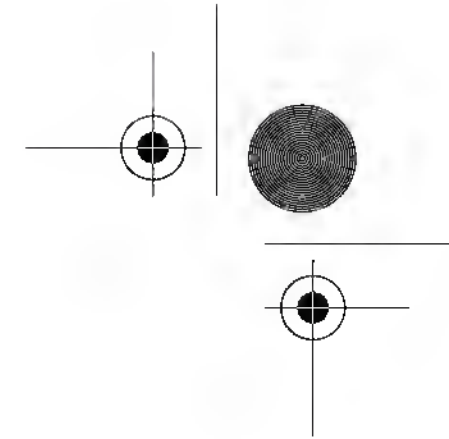
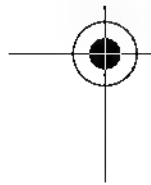
3.4.3 GROWTH/NO GROWTH INTERFACE MODELS

Microbial growth is restricted to nite ranges for any environmental factor, with growth rate sometimes declining abruptly within a very small increment of change of environment. Individual factor limits have been determined and collated (e.g., ICMSF, 1996a). That the growth range of microorganisms for one factor is reduced when a second environmental factor is less than optimal is also well recognized, and underlies the Hurdle concept (Leistner et al., 1985) also known as (multiple) barrier technology, or combined processes (Gorris, 2000). While the physiological basis of this synergy remains incompletely understood, the ability to de ne the limits to growth under combined environmental factors has enormous practical application in maintaining the microbial safety and quality of foods. Whether pathogens grow at all and the position of the G/NG boundary are of more interest than their growth rate because any growth implies a potential to cause harm to consumers. Similarly, so-called shelf-stable foods are sold, stored, and consumed over long periods of time. Therefore, the ability of spoilage organisms to grow at all implies that they have the potential to multiply to suf cient numbers to cause spoilage (Jenkins et al., 2000).

In the early to mid-1990s, a vein of experimentation using logistic regression techniques was begun with the aim of developing models that could de ne *absolute* limits to microbial growth in multifactorial space, irrespective of time of incubation or number of cells in the inoculum. One impetus for this research was the problem of listeriosis (Parente et al., 1998; Tienungoon et al., 2000). Strategies proposed to control the threat of listeriosis included “zero tolerance” (i.e., not detectable in a 25-g sample) of the presence of *L. monocytogenes* in foods *that could support its growth*, or to limit levels of contamination *at the point of consumption* to less than 100 cfu/g. Thus, foods that did not support the growth of *L. monocytogenes* were considered to pose signi cantly less risk and to require much less regulatory “attention” and testing. It was, therefore, of great commercial interest to be able to predict, without the need for protracted and expensive challenge testing, the potential for growth of specific bacteria within a particular food or, equivalently, product formulation options that would preclude growth.

Models de ning combinations of environmental conditions that *just* prevent growth have become known as “G/NG interface,” “growth boundary,” or more simply “growth limits” models. The importance of growth boundary models for the design of safe foods and setting of food safety regulations, for the design of shelf-stable





foods, and as a means of empowering the Hurdle concept by allowing it to be applied quantitatively has been discussed by various authors (Masana and Baranyi, 2000b; McMeekin et al., 2000; Ratkowsky and Ross, 1995; Schaffner and Labuza, 1997). Various approaches have been suggested to define the G/NG boundary. For convenience, these are discussed below under three broad groupings:

1. Empirical, deterministic, approaches
2. Logistic regression techniques
3. Artificial neural networks

Table 3.7 provides an overview of G/NG models published since 1990.

3.4.3.1 Deterministic Approaches

The first explicit definition of a microbial G/NG interface appears to be Pitt (1992), who derived regression equations from published data to describe the temperature/water activity interface for a toxin production and *Aspergillus* spp. growth. The equation used to describe the interaction between temperature and water activity limits for growth was:

$$T_g^{(\text{min-max})} = 29.27 \pm \sqrt{(856.71 - 2289 \times (1.172 - a_w))}$$

where $T_g^{(\text{min-max})}$ are the upper and lower temperature limits for growth at the specified water activity.

A similar equation was presented for a toxin production. The predicted interfaces from both models are shown in Figure 3.7.

To describe the pH/ $a_{w(\text{NaCl})}$ interface of the food spoilage organism *Brocothrix thermosphacta*, Masana and Baranyi (2000b) derived the midpoints of growth and no-growth observations by interpolation and fitted a polynomial function to those data. They noted that under some conditions, the interface was completely dominated by one factor or the other, so that their final model consisted of a pH vs. a_w parabolic curve and a NaCl-constant line. They also considered the effects of inoculum level on the interface, which was determined at 25°C for up to 24 days of incubation. Examples of the interface are shown in Figure 3.8.

Membré et al. (2001) estimated levels of sorbate that prevented growth of *Penicillium brevicompactum* in bakery products containing various levels of benzoate by extrapolation of kinetic data. Equations were derived to define growth-preventing combinations of sorbate and benzoate and were used to limit the range of predictions from the kinetic model they developed for *P. brevicompactum* growth rate.

Other workers have noted that the form and parameters of CPMs imply absolute limits to microbial growth, and suggested approaches to defining the G/NG interface based on estimates of cardinal parameters. In this vein Ratkowsky and Ross (1995), recognizing the relationship between absolute limits for each environmental factor and their relationship to the parameters of square-root-type models and CPMs, experimented with the use of a kinetic model as the basis of a growth boundary

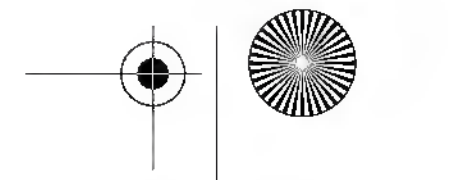
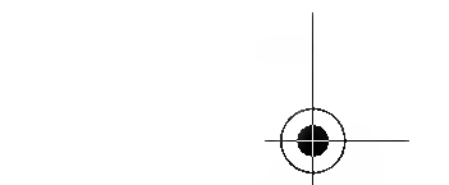
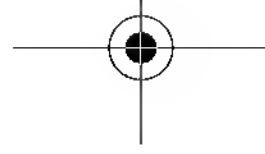
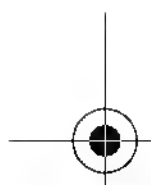


TABLE 3.7
Summary of Published Growth Boundary Models

| Reference | Organism | Strain | Medium | Environmental Factors | Ranges | | Levels | Replicates | Total Data Points | Measured by? | Time Limit | Other |
|-----------------------|-------------------------------|------------------------|---|------------------------|-----------------------|-----------------------|----------------|------------|-------------------|---|----------------|---|
| | | | | | Lower | Upper | | | | | | |
| Presser et al. (1998) | <i>Escherichia coli</i> | M23 (non-pathogenic) | Nutrient Broth | Temperature | 10 | 37 | 6 | 1 to 4 | 627 | OD Increase (con rmed as needed by culture) | 50 days | Linear logistic regression, SAS PROCNONLIN |
| | | | | a _w (NaCl) | 0.955 | 0.995 | 4 | | | | | |
| | | | | pH | 2.8 | 6.9 | ≥10 | | | | | |
| | | | | Lactic acid (mM) | 0 | 500 | 6 | | | | | |
| Parente et al. (1998) | <i>Listeria monocytogenes</i> | Scott A, V7, and L11 | Tryptone Soy Broth + 0.6% Yeast Extract | Nisin (IU/ml) | 1 | 2100 | | 5 | | | 7 days (@30°C) | Logistic regression with polynomial using LOGIT 1.14 module of Systat |
| | | | | Leucocidin F10 (AU/ml) | 1 | 2100 | | | | | | |
| | | | | pH | 4.7 | 6.5 | | | | | | |
| | | | | NaCl (% w/v) | 0.7 | 4.5 | | | | | | |
| | | | | EDTA (mmol) | 0.1 | 0.9 | | | | | | |
| | | | | Inoculum density | 1.6 × 10 ³ | 7.9 × 10 ⁷ | | | | | | |
| | | | | Nisin (IU/ml) | 8 | 200 | | | | | | |
| | | | | Leucocidin F10 (AU/ml) | 8 | 200 | | | | | | |
| | | | | pH | 4.7 | 6.5 | | | | | | |
| | | NaCl (% w/v) | 0.7 | 4.5 | | | | | | | | |
| | | EDTA (mmol) | 0.08 | 4.72 | | | | | | | | |
| | | Inoculum density | 0.6 × 10 ³ | 2.5 × 10 ⁷ | | | | | | | | |
| | | Nisin (IU/ml) | 50 | 250 | 10 | | 7 days (@30°C) | | | | | |
| | | Leucocidin F10 (AU/ml) | 1 | 250 | | | | | | | | |
| | | pH | 5.2 | 6 | | | | | | | | |
| NaCl (% w/v) | 1.8 | | | | | | | | | | | |
| EDTA (mmol) | 0.2 | 0.6 | | | | | | | | | | |
| Inoculum density | 1 × 10 ⁵ | | | | | | | | | | | |

TABLE 3.7 (Continued)
Summary of Published Growth Boundary Models

| Reference | Organism | Strain | Medium | Environmental Factors | Experimental Design | | | | Total Data Points | Measured by? | Time Limit | Other |
|-------------------------|---------------------------------|---|---|---|------------------------|-----------------------|------------------|-------------|-------------------|--------------|----------------|--|
| | | | | | Ranges | | Levels | Replicates | | | | |
| | | | | | Lower | Upper | | | | | | |
| Bolton and Frank (1999) | <i>Listeria monocytogenes</i> | Mixture (equal numbers) of Scott A, Brie 1, 71 Switzerland, 2379 LA | Soft fresh cheese (similar to "Mexican style" cheese) | Moisture (% w/w) salt (% w/w) pH | 42 2 5 | 60 8 6.5 | 4 4 6 | 3 | 288 | Viable count | 21 and 42 days | Binary or "ordinal" logistic regression using SAS PROC LOGISTIC with link functions. For the latter, three responses: P of growth, stasis, or death (according to change in viable count; $\pm 0.5 \log$ CFU) were modeled |
| Salter et al. (2000) | <i>Escherichia coli</i> | MR21 (STEC) | Nutrient Broth | Temperature a_w (NaCl) | 7.7 0.943 | 37 0.987 | 60 28 | 1-8, most 4 | 604 | OD increase | 50 days | Nonlinear logistic regression, SAS PROCLOGISTIC and PROCNONLIN |
| Jenkins et al. (2000) | <i>Zygosaccharomyces bailii</i> | 4637, history unknown | Acidic yeast nitrogen broth | Salt (NaCl, %w/v) Sugar (fructose, %w/v) Total acetic acid (%v/v) pH | 2.6 7 1.8 3.5 | 4.2 32 2.8 4 | 3 3 3 3 | 3 | 243 | OD increase | 29 days | SAS LIFEREG |

| | | | | | | | | | | | | |
|----------------------------|---|--|-------------------------------------|---|---|-----------------------------|----------------------|---|----------------------------|--|---------------|---|
| Tienungoon et al. (2000) | <i>Listeria monocytogenes</i> | (Scott A, L5 separate models) | TSB-YE | Temperature a _w (NaCl) pH Lactic acid (mM) | 3.1 0.928 3.7 0 | 36.2 0.995 7.8 500 | 30 60 10 14 | 1 to 4 | 2839 | OD increase | 90 days | Nonlinear logistic regression, SAS PROCLOGISTIC and PROCNONLIN |
| López-Malo et al. (2000) | <i>Saccharomyces cerevisiae</i> | Not stated Model based on data of Cerruti et al. (1990) | | a _w pH K-sorbate (ppm) | 0.93 3 0 | 0.97 6 1000 | 3 4 6 | | 72 (× 2 observation times) | Viable count, including decrease in viable count | 50 h or 350 h | Logistic regression with first-order polynomial using SPSS |
| Masana and Baranyi (2000b) | <i>Brocothrix thermosphacta</i> | MR 165 | Tryptone Soya Broth | NaCl pH Inoculum (cfu/350 µl) | 0.5 4.4 10 | 10 5.7 1 million | 11 7 3 | | | Viable count | | Polynomial fitted to midpoints of data-pairs of adjacent growth and no growth observed combinations |
| | | Fine grid experiments | | NaCl pH Inoculum (cfu/350 µl) | 45 combinations at 5 levels of NaCl and 5 levels of pH close to the G/NG boundary 10 1000 | | 2 | 10 | 450 | Viable count | | |
| Lanciotti et al. (2001) | <i>Bacillus cereus</i> <i>Staphylococcus aureus</i> <i>Salmonella enteritidis</i> | FG1 S33 B5 | BHI Broth BHI Broth BHI Broth | Temperature a _w (glycerol) pH Ethanol (% v/v) | 10 0.89 4 0 | 45 0.99 8 3 | 5 8 5 5 | 30 variables combinations over two independent trials for each organism | 2 × 30 for each strain | OD increase (600nm) | 2–7 days | Generalized linear logistic regression, Statistica (Statsoft) software |

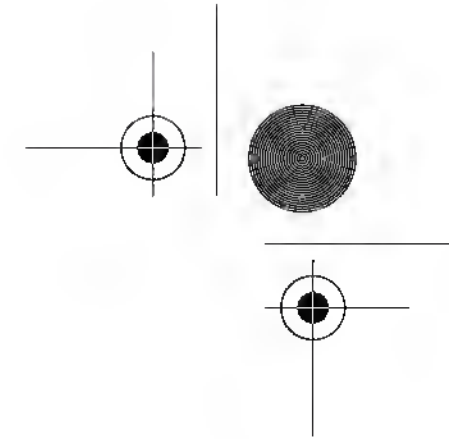
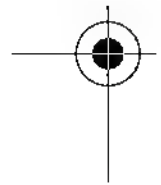
TABLE 3.7 (Continued)
Summary of Published Growth Boundary Models

| Reference | Organism | Strain | Medium | Environmental Factors | Experimental Design | | | | Total Data Points | Measured by? | Time Limit | Other |
|------------------------|--------------------------------|-------------------|-----------|---|------------------------------|---------------------------------|-----------------------|------------|-------------------|-------------------------------|------------|---|
| | | | | | Ranges | | Levels | Replicates | | | | |
| | | | | | Lower | Upper | | | | | | |
| Stewart et al. (2001) | <i>Staphylococcus aureus</i> | 5 strain cocktail | BHI Broth | a _w (glycerol) Initial pH K-sorbate (ppm) or Ca-propionate (ppm) Temperature (°C) | 0.95 4.5 0 37 | 0.84 7 1000 | 4 4 3 | 8 | 640 | OD increase | 168 days | Toxin assayed Modeled "time to growth" using LIFEREG |
| McKellar and Lu (2001) | <i>Escherichia coli</i> O15:H7 | 5 strain cocktail | TS Broth | Temperature (°C) Acetic acid (modeled as undissociated form) NaCl Sucrose pH | 10 0 0.50% 0 3.5 | 30 4% 16.50% 8% 6.0 | 5 8 8 3 6 | 5 | 1820 | Visible increase in turbidity | 3 days | Linear logistic regression used (polynomial form) |

| | | | | | | | | | | | | |
|------------------------------|--------------------------------|--|--|-----------------------------------|-------------|---------|-----|-----------------------------|--------------|---|--|--|
| Membré et al. (2001) | <i>Penicillium compactum</i> | Wild type from bakery products | MYSO agar | pH | 2.5 | 7.5 | ± 6 | 1 | 76 | Mycelial growth | 75 days | Not directly modeled, growth limits due to sodium benzoate and sorbic acid at pH 5 were derived by extrapolation of growth rate data |
| | | | | Sorbic acid | | | 1 | | | | | |
| | | | | Propionic acid | | | 1 | | | | | |
| | | | | Sodium benzoate | | | 1 | 5 | 122 | | | |
| | | | | pH | | | | | | | | |
| | | | | Sorbic acid (mg l ⁻¹) | 0.0 | 1000.0 | 6 | | | | | |
| | | | | Na-benzoate (mg l ⁻¹) | 0.0 | 300.0 | 4 | | | | | |
| | | | Commercial cakes | | | | | 6 | 4 | | | |
| Uljas et al. (2001) | <i>Escherichia coli</i> O15:H7 | 3 strain cocktail | Apple cider (juice) | pH | 3.1 | 4.3 | 7 | | 1600 × 3 | Turbidity (growth within 48 h at 35°C) after dilution of treated sample | 12 hours | SAS PROCLOGISTIC (dependency modeled as simple first order equations of predictor variables, no cross products) |
| | | | | Temperature | 5 | 35 | 4 | | | | | |
| | | | | Sodium benzoate | 0 | 0.1% | 3 | | | | | |
| | | | | Potassium sorbate | 0 | 0.1% | 3 | | | | | |
| | | | | Freeze-thaw | Not applied | Applied | 3 | 756 | | | | |
| | | | | Ciders type | | | 3 | 2 or 3 | | | | |
| <i>Staphylococcus aureus</i> | 5 strain cocktail | BHI Broth | a _w (NaCl, or sucrose-fructose) | | 0.84 | 0.95 | 4 | 8 | OD increase | 168 days | Toxin assayed Modeled "time to growth" using LIFEREG | |
| | | | | pH | 4.5 | 7 | 4 | | | | | |
| | | | | K-sorbate (ppm) | 0 | 1000 | 2 | | | | | |
| | | | | | | | | | | | | |
| <i>Listeria innocua</i> | ATCC 33090 | BHI Broth (+0.2% w/w glucose, +0.3% w/w yeast extract) | Temperature | | 0 | 43 | 16 | (pH constant) | Turbidimetry | 14 days | Novel term based on relative inhibition of growth rate-affecting factors data generated for combined kinetic model that predicts no growth (NLINFIT in MATLAB 5.2) | |
| | | | | pH | 4.5 | 9.4 | 15 | (temperature constant) | | | | |
| | | | | Propionic acid (mM) | 16 | 64 | 24 | (pH varied from 5 to 7.5) | | | | |
| | | | | Lactic acid (mM) | 20 | 138 | 27 | (pH varied from 4.8 to 7.1) | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |

TABLE 3.7 (Continued)
Summary of Published Growth Boundary Models

| Reference | Organism | Strain | Medium | Experimental Design | | | | | Total Data Points | Measured by? | Time Limit | Other |
|--|--|-------------------|--------------------------------------|-------------------------|--------|-------|--------|------------|-------------------|--------------------|------------------------------|--|
| | | | | Environmental Factors | Ranges | | Levels | Replicates | | | | |
| | | | | | Lower | Upper | | | | | | |
| Battey and Schaffner (2001) | Spoilage bacteria: <i>Acinetobacter calcoaceticus</i> and <i>Gluconobacter oxydans</i> | 2 strain cocktail | Cold lled, ready to drink, beverages | pH | 3 | 2.8 | 3.8 | 84 | | Viable plate count | 8 weeks at 25°C | Model is based on growth and inactivation rates. Included 14 duplicated validation trials (8 correctly predicted from model) |
| | | | | Titrateable acidity (%) | 3 | 0.2 | 0.6 | | | | | |
| | | | | Sodium benzoate (ppm) | 3 | 100 | 350 | | | | | |
| | | | | Sugar content (°C Brix) | 3 | 8 | 16 | | | | | |
| | | | | Potassium sorbate (pp) | 3 | 100 | 350 | | | | | |
| Battey et al. (2002) | Spoilage yeasts: <i>Saccharomyces cerevisiae</i> , <i>Zygosaccharomyces bailii</i> , <i>Candida lipolytica</i> | 3 strain cocktail | Cold lled, ready to drink, beverages | pH | 3 | 2.8 | 3.8 | 84 | | Viable plate count | 8 weeks at 25°C | Included 14 duplicated validation trials (all correctly predicted from model) |
| | | | | Titrateable acidity (%) | 3 | 0.2 | 0.6 | | | | | |
| | | | | Sodium benzoate (ppm) | 3 | 100 | 350 | | | | | |
| | | | | Sugar content (°C Brix) | 3 | 8 | 16 | | | | | |
| | | | | Potassium sorbate (pp) | 3 | 100 | 350 | | | | | |
| Hajmeer and Basheer (2002, 2003a, 2003b) | Data of Salter et al. (2000), see above | | | | | | | | | | Probabilistic Neural Network | |



model using linear logistic regression. This approach is discussed further below in Section 3.4.3.2.

The approaches of Augustin and Carrier (2000b) and Le Marc et al. (2002) were presented in Section 3.2.3. Essentially, these approaches are empirical. They are based on assumed interactions between factors and are not *fitted* to G/NG data. An example of the response predicted by these approaches is shown in Figure 3.11.

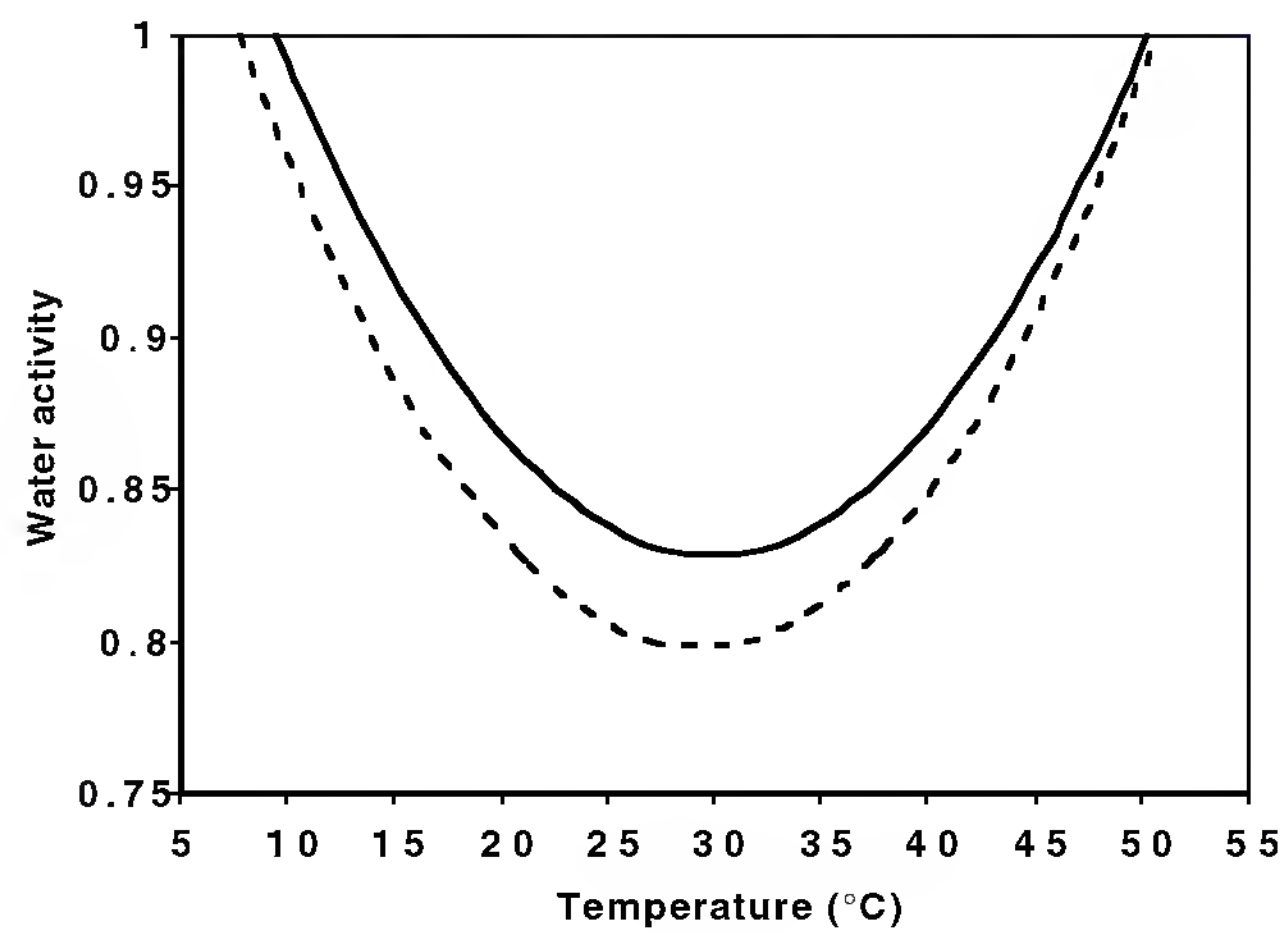


FIGURE 3.7 Predicted temperature–water activity interface for mold (*Aspergillus* spp.) growth (dashed line) and a atoxin production (solid line) from the model of Pitt (1992).

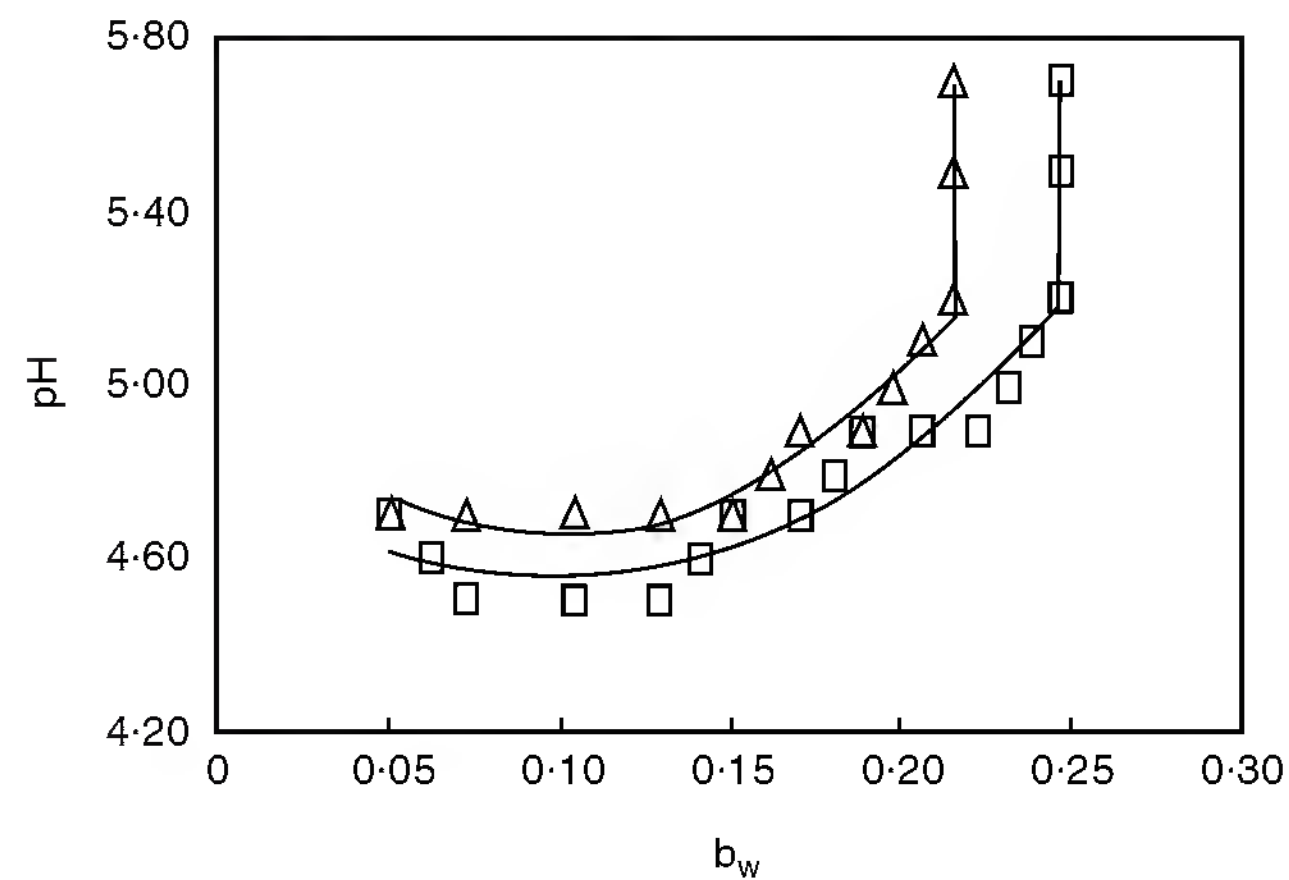
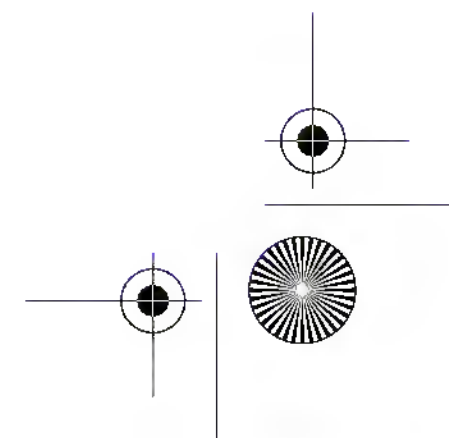
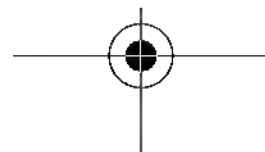
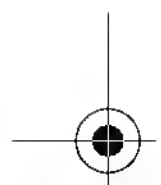


FIGURE 3.8 Data and modeled growth/no-growth boundary for *Brochothrix thermosphacta* in response to pH and water activity at 25°C. Water activity data were rescaled to $b_w = \sqrt{1 - a_w}$. The data are for an inoculum of 1.5×10^6 cells/well (□), or for an inoculum of 1.5×10^1 and 1.5×10^3 cells/well (Δ). (Reproduced from Masana, M.O. and Baranyi, J. *Food Microbiol.*, 17, 485–493, 2000b. With permission.)



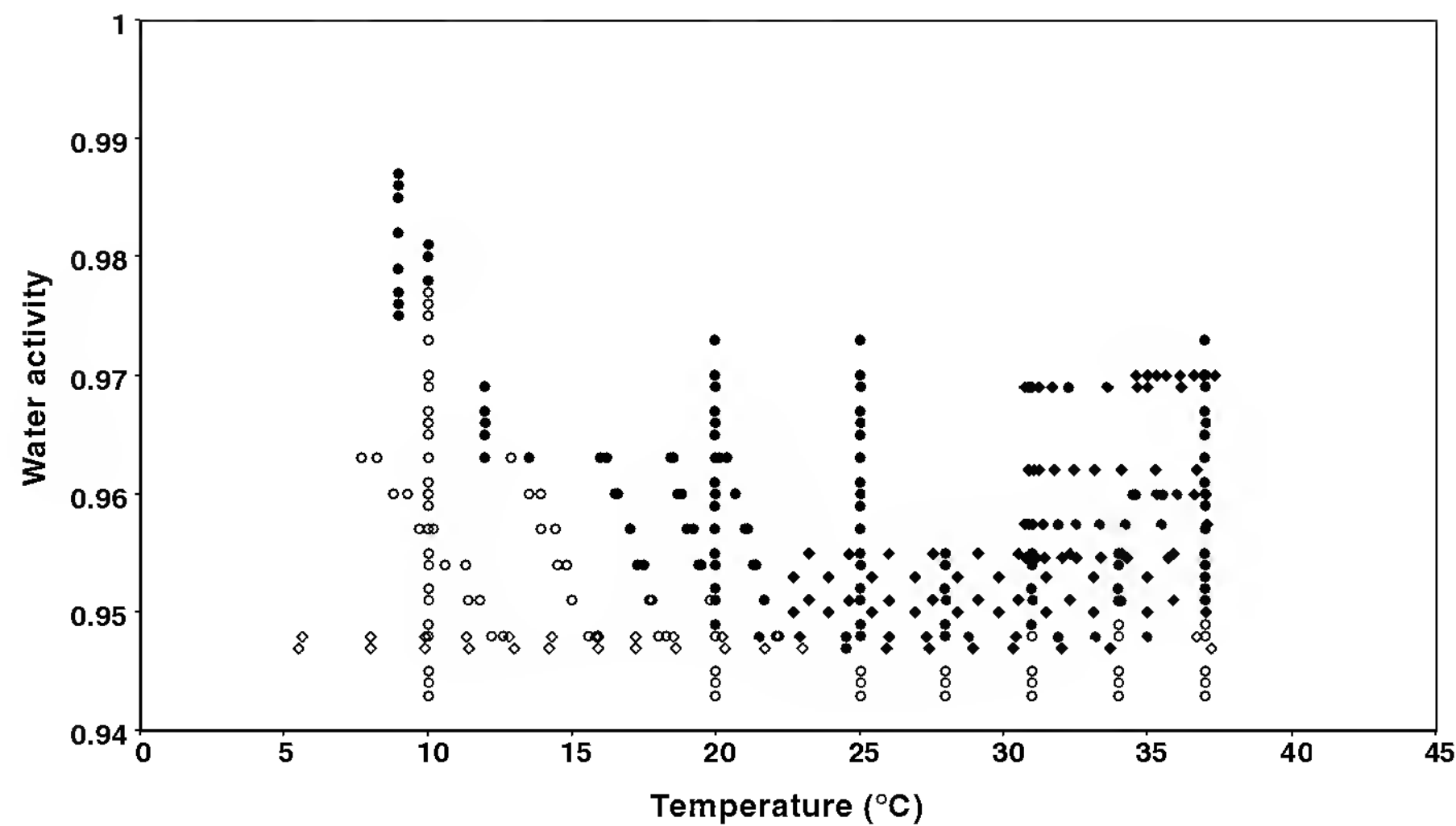
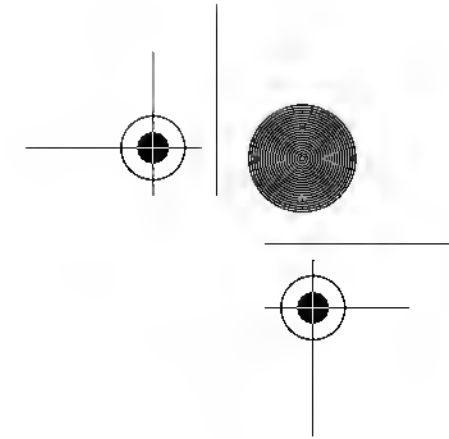
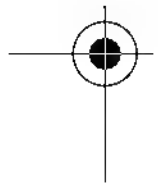
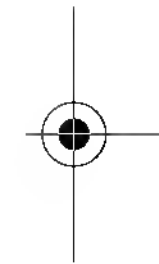
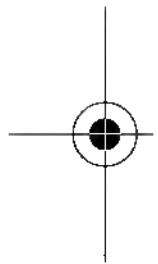
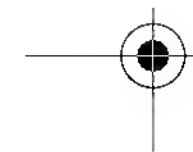
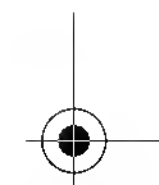
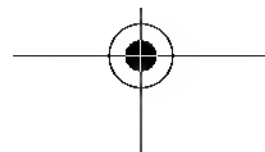
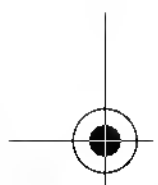


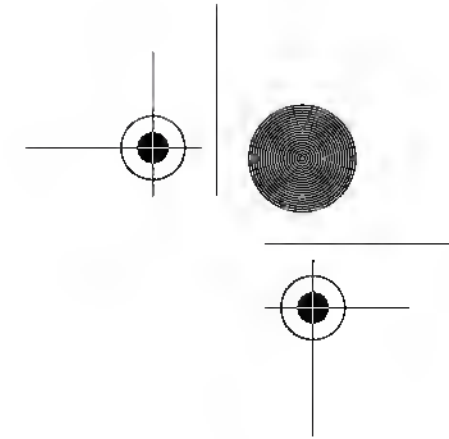
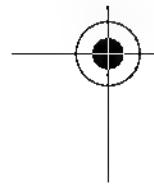
FIGURE 3.9 Data obtained from separate experiments for the growth/no-growth (G/NG) boundary of *Escherichia coli*. Data are from Salter et al. (2000) (circles) and from unpublished results of the authors (diamonds). Near the G/NG boundary, the data obtained from discrete experiments do not form a smooth (monotonic) boundary, suggesting that small differences in experimental procedures can significantly affect the position of the boundary. Open symbols denote no-growth conditions, and solid symbols indicate that growth was observed.



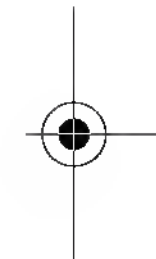
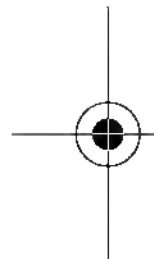
The above approaches can be considered to be deterministic; i.e., they predict only one position ($P_{\text{growth}} = 0.5$) for the boundary, although the position of boundaries can be adjusted by “weighting” data in the case of Masana and Baranyi (2000b) or by selecting an appropriate value for θ in the case of the Le Marc et al. (2002) approach (see Section 3.2.3). While the data of Masana and Baranyi (2000b) included tenfold replication, the midpoints of the *most* “extreme” conditions that *did* allow growth, and the *least* “extreme” conditions that *did not* allow growth were estimated by interpolation and considered to represent 50% probability of growth. Other workers have suggested that some problems require higher levels of confidence that growth will not occur, so that methods that enable definition of the interface at selected levels of statistical confidence may have greater utility.

Another approach that implicitly characterizes the G/NG interface is that of combined growth and death models in which the rate of growth and rate of death under specified conditions are estimated simultaneously. The G/NG interface can be inferred from those combinations of conditions where growth rate and death rate are equal (see, e.g., Jones and Walker, 1993; Jones et al., 1994). A similar approach is evident in Battey et al. (2001) who modeled both the rates of growth and rates of death of spoilage molds in ready to drink beverages. The G/NG interface was given, implicitly, as that set of conditions where the rate of growth was equal to the rate of death.





Ratkowsky et al. (1991) noted that as environmental conditions become more inhibitory to microbial growth the variability in growth rates increases widely, which implies that the probability that growth occurs at all becomes uncertain, because the left-hand tail of the growth rate distribution falls below zero. This is supported in the results of Whiting and colleagues (Whiting and Call, 1993; Whiting and Oriente, 1997; Whiting and Strobaugh, 1998), where P_{\max} (the proportion of spores that successfully germinated and initiated growth) was shown to decline at increasingly stringent conditions. Conversely, Masana and Baranyi (2000b) observed, as have other workers (McKellar and Lu, 2001; Presser et al., 1998; Salter et al., 2000; Tienungoon et al., 2000), that the difference in conditions that allow growth and those that do not is usually abrupt, and often at or beyond the limits of resolution of instruments commonly used to measure such differences. Thus, Masana and Baranyi (2000b) questioned the need for approaches that model the transition between conditions leading to high probability of growth and those leading to low probabilities of growth. While this abrupt transition appears consistent *within* replicated experiments it is less certain, however, that the same consistency is true *between* experiments. Figure 3.9, showing experimental data, suggests that responses near the boundary may be inconsistent when data from several discrete experiments are combined. This may suggest subtle, but important, differences in response related to the physiology of the inoculum, or its concentration. Furthermore, it suggests that the ability to characterize probabilities of growth under specified sets of conditions may be an important element of growth boundary models and that the boundary may not be “absolute” but depend on the physiological state of the cell and, by inference, on the size of the inoculum. This will be discussed further in Section 3.4.4.

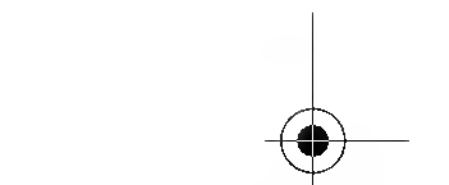
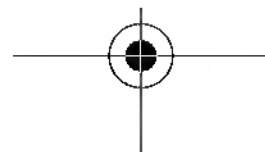
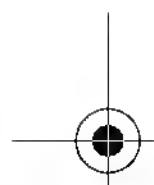


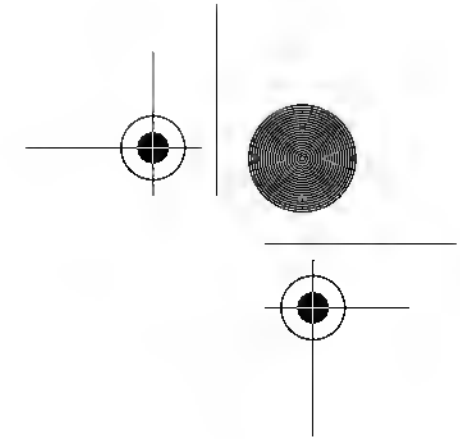
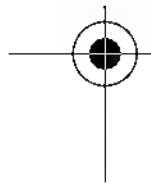
3.4.3.2 Logistic Regression

Ratkowsky and Ross (1995) and others (Bolton and Frank, 1999; Jenkins et al., 2000; Lanciotti et al., 2001; López-Malo et al., 2000; McKellar and Lu, 2001; Parente et al., 1998; Stewart et al., 2001, 2002; Uljas et al., 2001) reintroduced the use of logistic regression to model categorical data (i.e., growth or no growth) in predictive microbiology, enabling probabilistic determination of the G/NG boundary. Use of the logit function enabled the probability of growth under specific sets of conditions to be estimated, so that the G/NG boundary could be specified at selected levels of confidence.

Ratkowsky and Ross (1995) aimed to model absolute limits to growth in multifactorial space, but only had available data based on a 72-h observation period. While most other workers have preferred polynomial functions to describe the effect of independent variables on the logit function, in the former approach a square-root-type kinetic model was ln-transformed and used as the basis of the function relating the logit of probability of growth to independent variables, e.g., temperature, water activity, pH. This approach was adopted in an attempt to retain some level of biological interpretability of the models, a desire echoed by others (Augustin and Carlier, 2000a,b; Le Marc et al., 2002).

The form of the G/NG interface model of Presser et al. (1998) was derived from the kinetic model of Presser et al. (1997) for the growth rate of *E. coli* (see Equation





3.10). Novel data were generated specifically to assess the limits of *E. coli* growth under combinations of temperature, pH, a_w , and lactic acid. The corresponding G/NG model had the form:

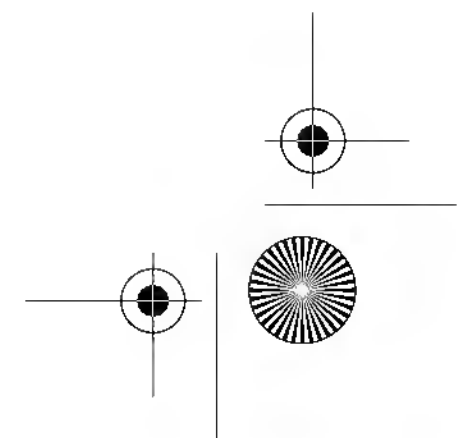
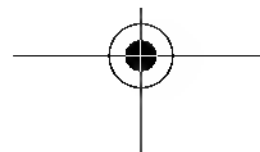
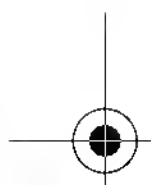
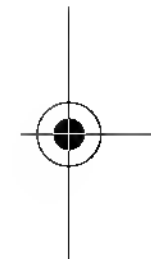
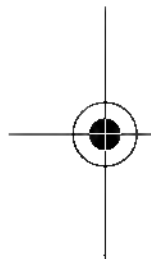
$$\begin{aligned} \text{LogitP} = & 28.0 + 8.90 \ln(a_w - 0.943) \\ & + 2.0 \ln(T - 4.00) + 4.59 \ln(1 - 10^{3.9 - \text{pH}}) \\ & + 6.96 \ln[1 - LAC / (10.7 \times (1 + 10^{\text{pH} - 3.86}))] \\ & + 3.06 \ln[1 - LAC / (823 \times (1 + 10^{3.86 - \text{pH}}))] \end{aligned} \quad (3.44)$$

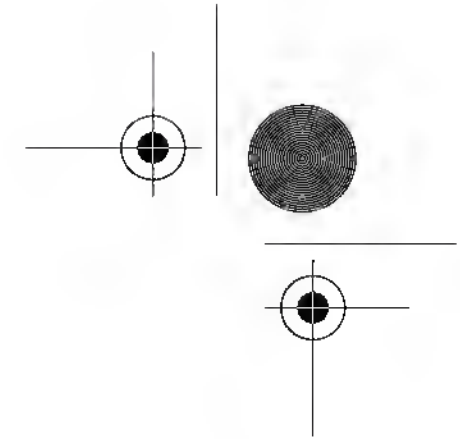
where all terms are as defined in Section 3.2.1.

Some parameters in that model had to be determined independently, i.e., were not determined in the regression, and were derived from the fitted values of square-root-type kinetic models. Essentially the same approach was adopted by Lanciotti et al. (2001) to develop G/NG models for *B. cereus*, *S. aureus*, and *Salmonella enteritidis*. Ratkowsky (2002) commented on the increased flexibility in being able to determine all of the parameters in the model during the regression, and subsequent studies developed the approach, eventually leading to a novel nonlinear logistic regression technique (Salter et al., 2000; Tienungoon et al., 2000). Ratkowsky (2002) pointed out that nonlinear logistic regression was a new statistical technique and discussed benefits and problems with that approach specifically in relation to growth limits modeling. A problem with models of the form of Equation 3.44 is that for conditions more extreme than the parameters corresponding to T_{\min} , pH_{\min} , $a_{w \min}$, etc., and which are tested experimentally though not expected to permit growth, the terms containing those parameters would become negative. As all of those terms are associated with a logarithmic transformation, the expression cannot be calculated during the regression and such values are ignored in the model fitting process, or have to be eliminated from the data set before the fitting process begins. This, in turn, affects the values of the parameters of the fitted model. Ratkowsky (2002) comments that an objective method for selection and deletion of such data is necessary, but does not yet exist.

Bolton and Frank (1999) extended the binary logistic regression approach by recoding growth and no growth data to allow a third category: survival, or stasis. They termed this approach ordinal logistic regression. Parente et al. (1998) “reversed” the response variable, and applied logistic regression techniques to the probability of survival/no survival of *L. monocytogenes* in response to bacteriocins, pH, EDTA, and NaCl. Stewart et al. (2001) modeled the probability of growth of *S. aureus* within 6 months of incubation at 37°C, and at reduced water activity achieved by various humectants. They also compared the growth boundary with the boundary for enterotoxin production, and observed a close correlation between the two criteria.

Growth limits models have also been developed for spoilage organisms including *Saccharomyces cerevisiae* (López-Malo et al., 2000) and *Zygosaccharomyces bailii* (Jenkins et al., 2000) and cocktails of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Candida lipolytica* (Battey et al., 2002). Interestingly, the study of Jenkins et al. (2000), while encompassing broader ranges of factor combinations, confirmed





the simpler and earlier model of Tuynenburg Muys (1971). That model, which specifies combinations of molar salt plus sugar and percent undissociated acetic acid for stability of acidic sauces, still forms the basis of the industry standard for those products. This observation suggests that limits to growth under combined conditions can be highly reproducible.

3.4.3.3 Relationship to the Minimum Convex Polyhedron Approach

The concept of the MCP was introduced by Baranyi et al. (1996) (see Figure 3.10) to describe the multifactor “space” that just encloses the interpolation region of a predictive kinetic model. If the interpolation region exactly matched the growth region of the organism then the MCP would also describe the growth limits of the organism. In practice, however, it would be impossible to undertake sufficient measurements to completely define the MCP; i.e., the MCP has “sharp” edges because of the method of its calculation, whereas from available studies (see Figures 3.8 and 3.9) the G/NG interface forms a continuously curved surface. However, it might also be possible to use no-growth data to create a no-growth MCP and to combine the growth MCP and no-growth MCP to define a region within which the G/NG boundary must lie. This approach has been assessed and compared to a model of the form of Equation 3.43 by Le Marc and colleagues (Le Marc et al., 2003). These workers concluded that the logistic regression modeling approach produced a smoother response surface, more consistent with observations, but that the MCP approach had the advantage of being directly linked to observations and therefore was not a prediction from a model.

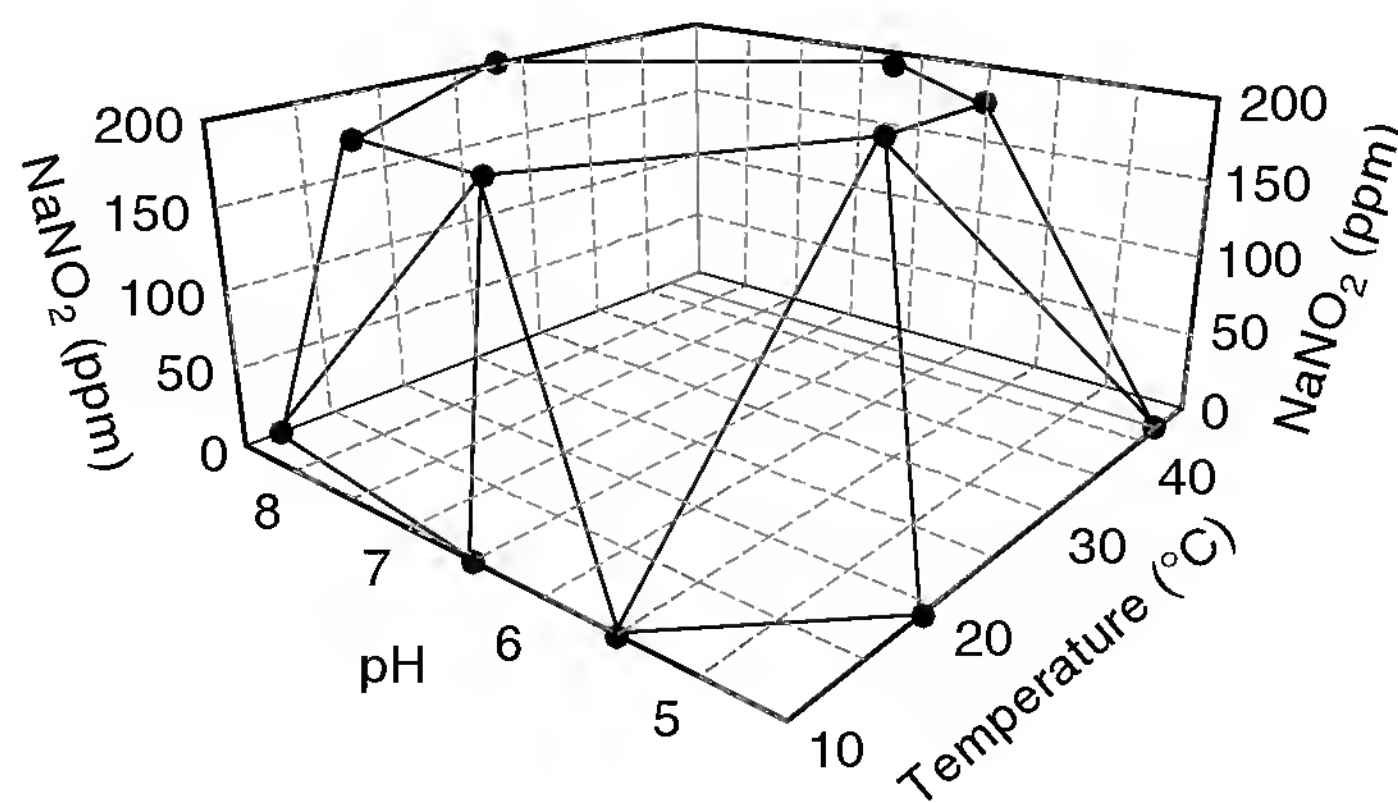
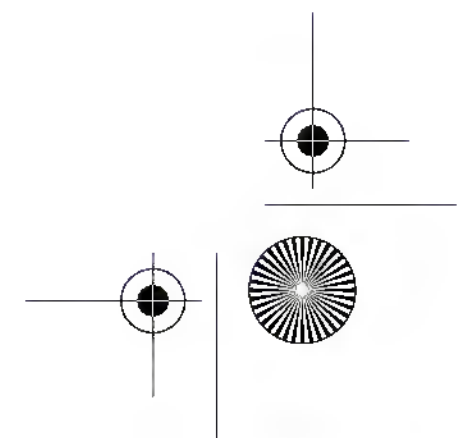
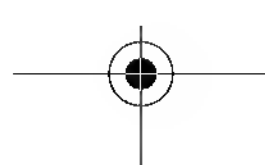
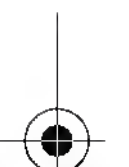
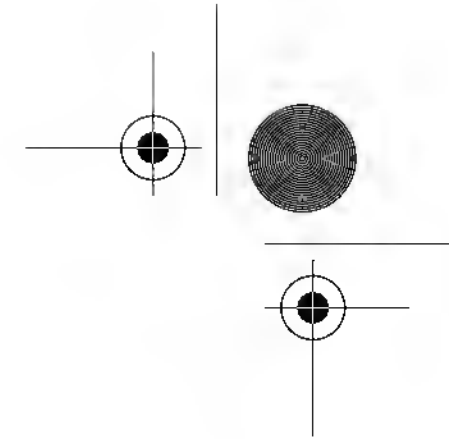
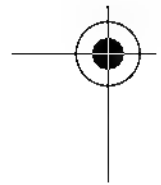


FIGURE 3.10 Interpolation region (MCP) for a model that includes four-factor combinations (T , pH, NaCl, NaNO_2). The interpolation region shown is that for NaCl = 0.5%, but is based on the complete data set. Solid circles indicate conditions under which observations have been made, while the lines represent the edges of the MCP. (From Masana, M.O. and Baranyi, J. *Food Microbiol.*, 17, 367–374, 2000a. With permission.)





3.4.3.4 Artificial Neural Networks

Recently, Hajmeer and Basheer (2002, 2003a,b) demonstrated the use of a Probabilistic Neural Network (PNN) approach to definition of the G/NG interface. PNNs are a form of ANN (see Section 3.2.6). In a series of papers, based on modeling the data of Salter et al. (2000) for the effects of temperature and water activity (due to NaCl) on the growth limits of *E. coli*, Hajmeer and Basheer concluded that their PNN models provided a better description of the data of Salter et al. (2000) than did the nonlinear logistic regression method referred to above. Their conclusion is considered in more detail in Section 3.4.3.5 below.

It should be noted that neither the logistic regression models described above, nor the PNN, produce an equation that describes the interface. Rather, the output of those models is the probability that a given set of conditions will allow growth. To define the interface, it is necessary to rearrange the model for some selected value of P to generate an equation that describes the G/NG boundary.

3.4.3.5 Evaluation of Goodness of Fit and Comparison of Models

Methods for evaluation of performance of logistic regression models include the receiver operating curve (ROC; also referred to as the concordance rate), the Hosmer–Lemeshow goodness-of-fit statistic, and the maximum rescaled R^2 statistic. These are considered in greater detail in Tienungoon et al. (2000).

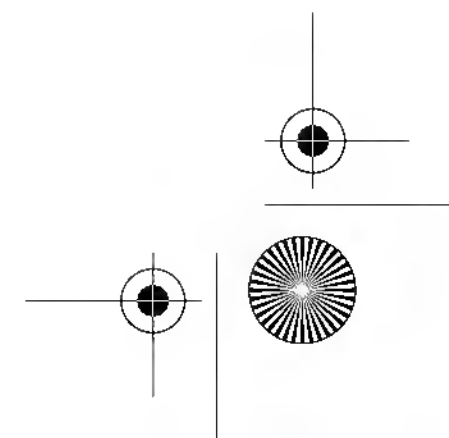
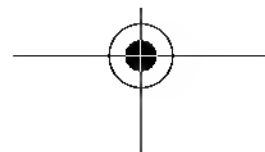
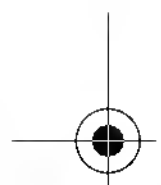
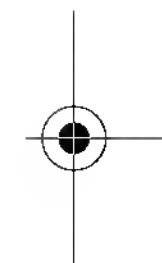
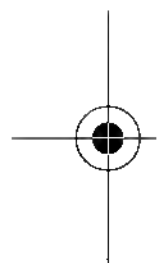
Briefly, the ROC is obtained from the proportion of events that were correctly predicted compared to the proportion of nonevents that were correctly predicted. The closer the value is to 1, the better the level of discrimination. In epidemiological studies, ROC values > 0.8 are considered excellent. ROC values for G/NG models are typically much higher.

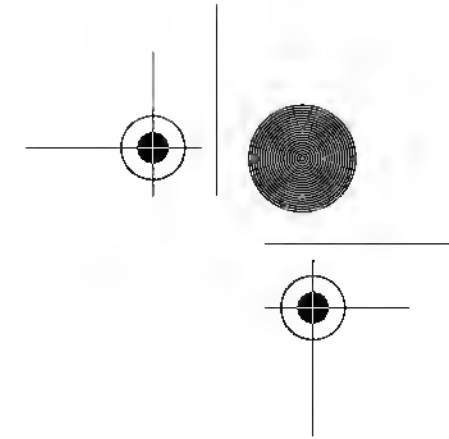
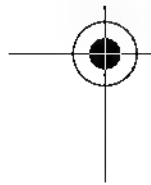
The Hosmer–Lemeshow index involves grouping objects into a contingency table and calculating a Pearson chi-square statistic. Small values of the index indicate a good fit of the model.

The maximum rescaled R^2 value is proposed for use with binomial error as an analogy to the R^2 value used with normally distributed error. The closer the value is to 1, the greater is the success of the model in predicting the observed outcome from the independent variables. Zhao et al. (2001) cite the deviance test and graphical tools such as the index plot and half normal plot as methods for determining goodness of fit of linear logistic regression models.

Other methods based on calculation of indices from the “confusion matrix” (Hajmeer and Basheer, 2002, 2003b) or the equivalent “contingency matrix” (Hajmeer and Basheer, 2003a) were used to compare performance between models derived from different approaches and applied to the same data.

Another method of evaluation is to compare the fitted model to independent data sets (Bolton and Frank, 1999; Masana and Baranyi, 2000b; Tienungoon et al., 2000) although, generally, such data are not readily available (see, e.g., McKellar and Lu, 2001). The model of Tienungoon et al. (2000) for *L. monocytogenes* growth boundaries showed very good agreement with the data of McClure et al. (1989) and George



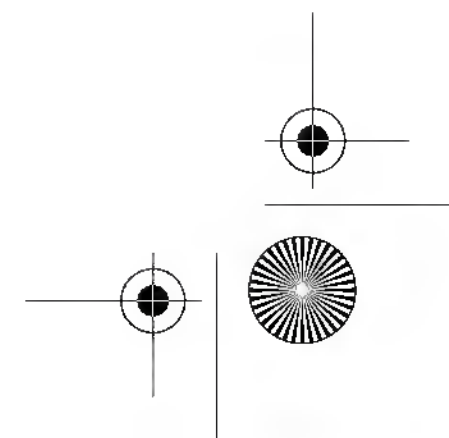
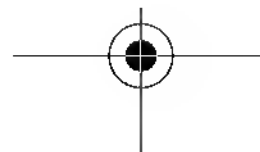
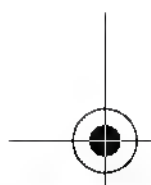
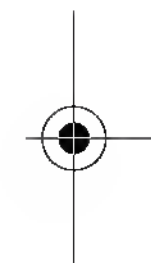
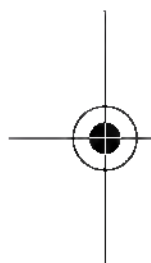


et al. (1988) despite that different strains were involved. There is also a remarkable level of similarity between the observations of Tienungoon et al. (2000) and the observations of Le Marc et al. (2002) on growth limits of *L. innocua*. Several publications, however, report growth of *L. monocytogenes* at temperatures lower than the minimum growth limit predicted by the Tienungoon et al. (2000) model, possibly indicating strain variation or that the experimental design failed to recognize important elements that facilitate *L. monocytogenes* growth at temperatures $< 3^{\circ}\text{C}$, i.e., that an inappropriate growth substrate was used. Similarly, McKellar and Lu (2001) reported that their model failed to predict growth of *E. coli* O157:H7 under conditions where it had been previously reported, although it should be noted that their model was limited to observation of growth within 72 h. Bolton and Frank (1999) compared the predictions of their growth limits models for *L. monocytogenes* in cheese to the data of Genigeorgis et al. (1991) for *L. monocytogenes* growth in market cheese. The models predicted correctly in 65% of trials (42-day model) and 81% of trials (21-day model).

Given the diversity of approaches, it is pertinent to ask: does one method for defining the G/NG interface perform better than another? As with kinetic models, the ability to describe a specific experimental data set does not necessarily reflect the ability to predict accurately the probability of growth under novel sets of conditions. While measures of performance of logistic regression models are available, they can be readily affected by the data set used. Perfect agreement between observed and modeled data responses may not be possible if there are anomalies in the data. Figure 3.11 provides a clear example. Nonetheless, for many growth limits models high rates of concordance (typically $>90\%$) have been reported. As noted earlier, in epidemiological logistic regression modeling, rates higher than 70% are considered to represent good fits to the data, implying that the limits to microbial growth are highly reproducible when well-controlled experiments are conducted.

To date, only one direct comparison of G/NG modeling approaches has been presented (Hajmeer and Basheer, 2002, 2003a,b) but this was based on one data set only, i.e., that of Salter et al. (2000) for the growth limits of *E. coli* in temperature/water activity space. Only by comparing the performance of different modeling approaches applied to multiple data sets does an appreciation of overall model performance emerge. Nonetheless, to illustrate differences between models and give some appreciation of their overall performance we compare several models using the data of Salter et al. (2000) for the growth limits of *E. coli* R31 in response to temperature and water activity. The model types compared are:

1. The PNN of Hajmeer and Basheer (2003a), which those authors were able to summarize as a relatively simple equation
2. A model of the type of Equation 3.44 fitted to a subset of the Salter et al. (2000) data set by Hajmeer and Basheer (2003a) (It should be noted that, contrary to what is stated in that publication, the model presented by Hajmeer and Basheer was not generated by nonlinear logistic regression but by a two-step linear logistic regression as described in Presser et al., 1998)



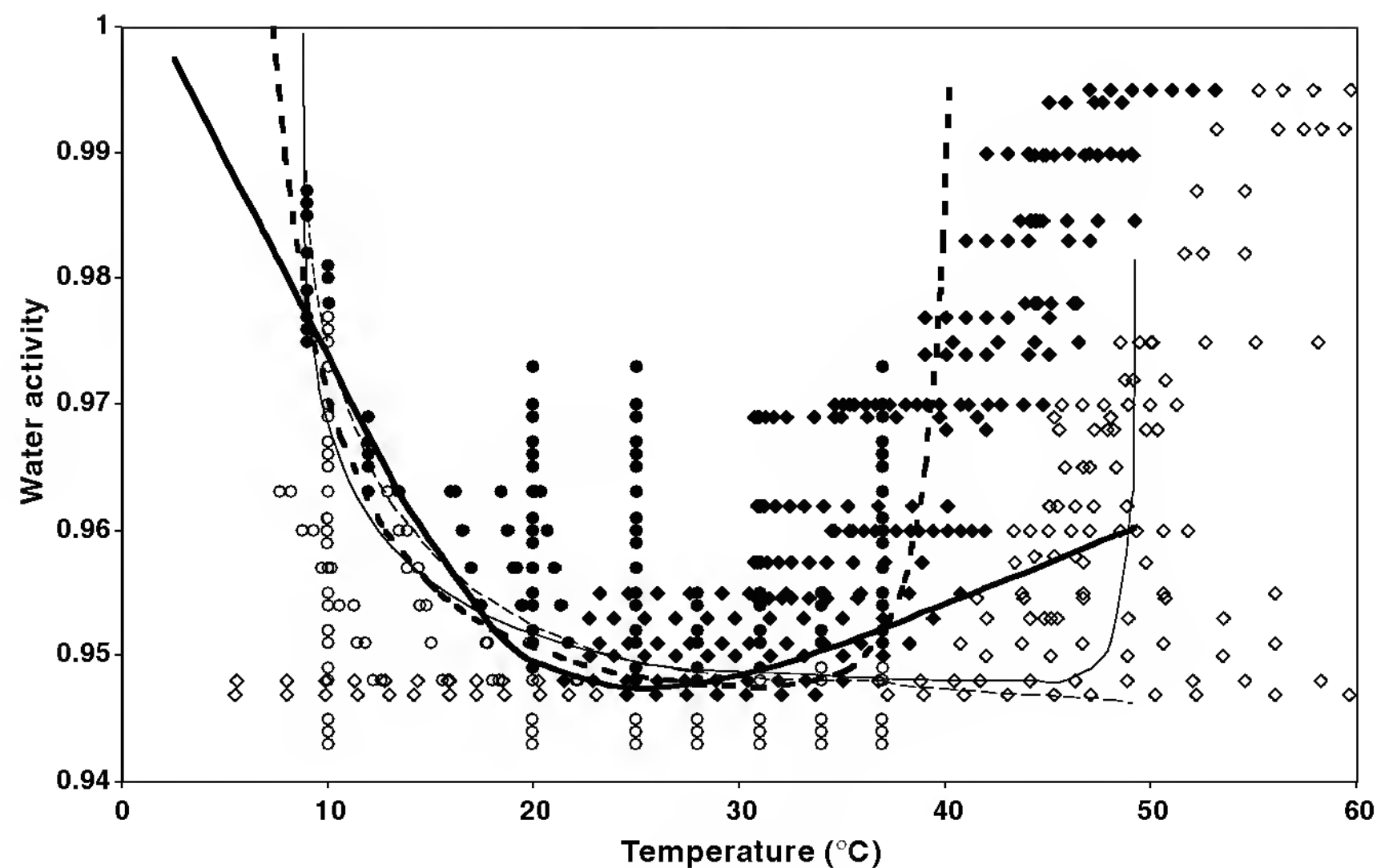
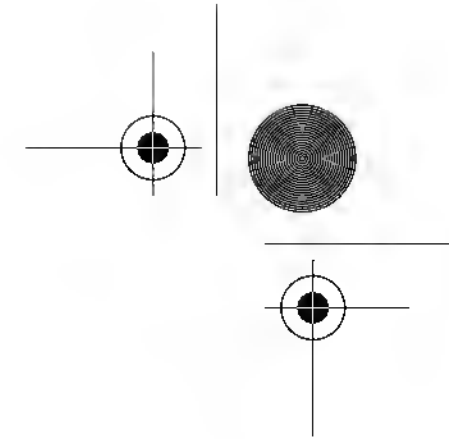
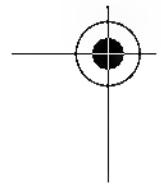


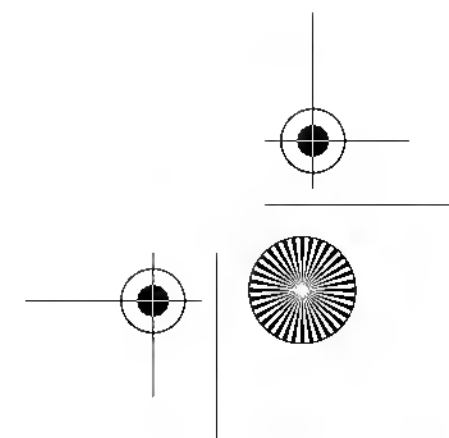
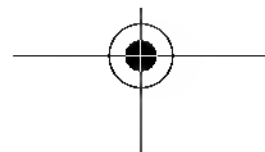
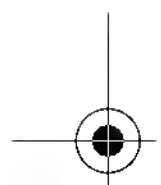
FIGURE 3.11 Comparison of predicted no growth boundaries for four modeling approaches applied to the data of Salter et al. (2000) (circles) for the growth limits of *Escherichia coli* R31 in response to temperature and water activity (NaCl) combinations. Approach of Hajmeer and Basheer PNN (heavy solid line); Linear Logistic /Equation 3.44 (heavy dashed line); Le Marc et al. (2002) (light solid line); Augustin and Carlier (2000a) (light dashed line). The data set was subsequently augmented with new data (diamonds), which reveals that none of the models extrapolate reliably. (Solid symbols: growth; open symbols: no growth.)

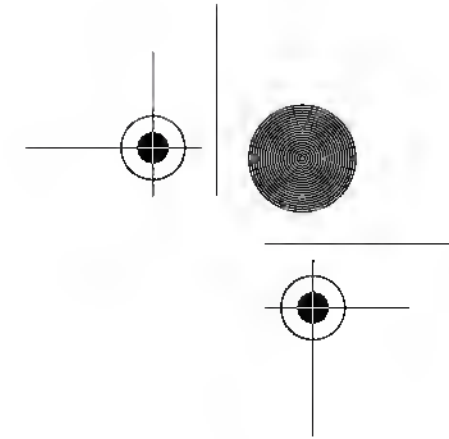
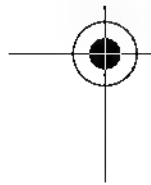
3. A model of the type of Le Marc et al. (2002; Equation 3.25 to Equation 3.27), where $T_{\max} = 49.23^{\circ}\text{C}$ (to be consistent with the logistic regression model parameter), $a_{w\min} = 0.948$, and $T_{\min} = 8.8^{\circ}\text{C}$, based on the minimum water activity and temperature, respectively, at which growth were observed
4. A model of the type of Augustin and Carlier (2000b; Equation 3.24) assuming that $T_{\min} = 8.8^{\circ}\text{C}$ and $a_{w\min} = 0.948$, consistent with the parameter values used for the Le Marc et al. (2002) model

The predicted interfaces are shown in Figure 3.11, together with the data used to generate the models. (Note that the subsets of 143 of the 179 data of Salter et al. (2000) used by Hajmeer and Basheer (2003a) to fit the PNN and the Equation 3.44 type model were not identified.)

When compared to the full data set, the level of misprediction ranged from ~15 to 20 of the 179 data points for each of the models, suggesting that the level of performance was not greatly different despite very different modeling approaches.* A complication in the comparison of G/NG model performance is that most of the

* It should be noted that this analysis disagrees with the results of Hajmeer and Basheer (2002) who reported only two to four mispredictions for the total (i.e., training and validation) data set.





data are readily predicted, e.g., those that fall outside the known limits for growth for individual environmental factors when all other factors are optimal. Such data can “overwhelm” the data in which one is most interested, i.e., in the relatively narrow region where factors interact to reduce the biokinetic ranges and, yet more specifically, where the probability of growth rapidly changes from “growth is very likely” to “growth is very unlikely.” These data define the interface and, consequently, data closest to the interface are more important when comparing model performance. This has implications for experimental design, as discussed in Section 3.4.4 below.

To assess whether one model might be preferred on theoretical grounds, as adjudged by its ability to extrapolate reliably, the predictions of all models in the temperature range above 35°C can be compared to data subsequently generated, shown in Figure 3.11, and not used to generate the models. Clearly, none of the models extrapolate well.

From the above comparison, it appears that despite very different modeling approaches and degrees of complexity of modeling, there is currently little to differentiate those approaches on the basis of their ability to describe the G/NG interface or on their ability to predict outside the interpolation region.

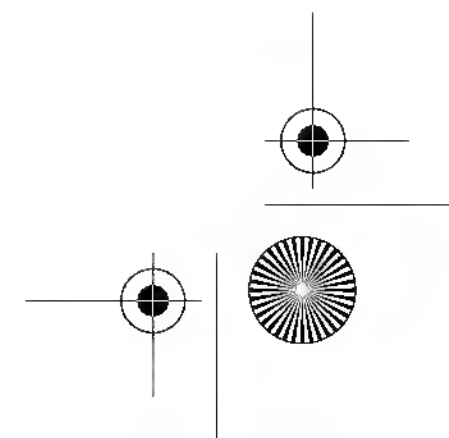
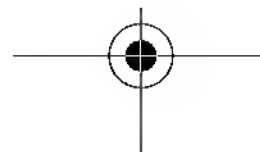
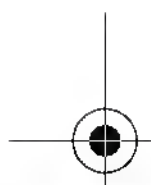
3.4.4 EXPERIMENTAL METHODS AND DESIGN CONSIDERATIONS

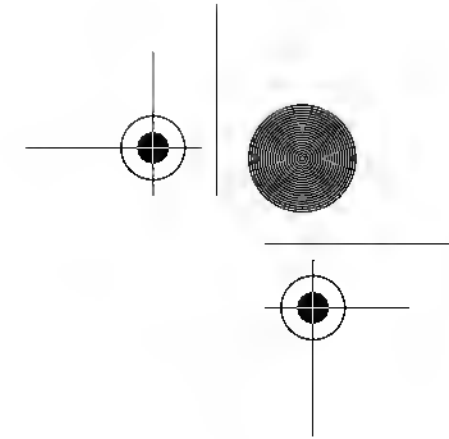
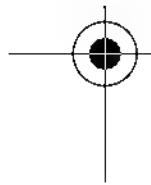
As suggested above, currently there is little mechanistic understanding of how environmental factors interact to prevent bacterial growth and it must be recognized as a possibility that there is no single, common mechanism underlying the observed boundaries for different factor combinations. Consequently, it is not possible from first principles to design the optimal experiment that captures the essential information that will characterize the response and lead to reliable models. Instead, at this time, experimental methods must be focused toward gaining enough data in the interface region to be able to describe empirically the limits to growth.

First of all, two approaches may be distinguished that could affect the experimental methods chosen. In one, the interest is in whether growth/toxin production, etc. is possible within some specified time limit, which may be related to the shelf life of the product. In other approaches, the objective is to define absolute limits to growth, i.e., the most extreme combinations of factors that just allow growth. McKellar and Lu (2001) argue that there is always a time limit imposed on G/NG modeling studies. Strategies exist, however, that provide greater confidence that the “absolute” limits to growth are being measured. Some of these are discussed below.

3.4.4.1 Measuring Both Growth and Inactivation

Several groups have assessed both growth and inactivation in their experimental treatments (McKellar and Lu, 2001; Parente et al., 1998; Presser et al., 1999; Razavilar and Genigeorgis, 1998). In this way the position of the boundary is inferred from two “directions.” If growth is not observed, an observer cannot be sure whether growth is not possible or has not occurred *yet*. If it is known that at some more extreme condition inactivation occurs, it can be inferred that the G/NG boundary lies between those two sets of conditions.





A potential problem with this strategy is that cultures can initially display some loss of viability, but with survivors eventually initiating growth; i.e., population decline cannot unambiguously be interpreted as “growth is not possible.” Numerous studies (e.g., Mellefont et al., 2003) have demonstrated that rapid transfer of a culture from one set of conditions to another that is more stressful can induce injury and death, but that survivors will eventually adjust and be able to grow. This has been termed the Phoenix phenomenon (Shoemaker and Pierson, 1976). Such regrowth has been reported in the context of G/NG modeling (Bolton and Frank, 1999; Masana and Baranyi, 2000b; Parente et al., 1998; Tienungoon et al., 2000).

Clearly, an experimenter interested in determining the “absolute” G/NG boundary will need to maximize the resistance of the inoculum to stress on exposure to the new, more stressful, environment. The use of stationary phase cultures as inocula would seem to be a minimum requirement. It may be necessary to habituate cultures to the test conditions (e.g., growth at conditions just less harsh) prior to inoculation into the test conditions to maximize the chance that growth, if possible, will be observed. One way to maximize the likelihood of observing the most extreme growth limits would be to use cultures growing at the apparent limits as inocula into slightly more stringent conditions. This also has the advantage of minimizing growth lags on inoculation into a harsher environment.

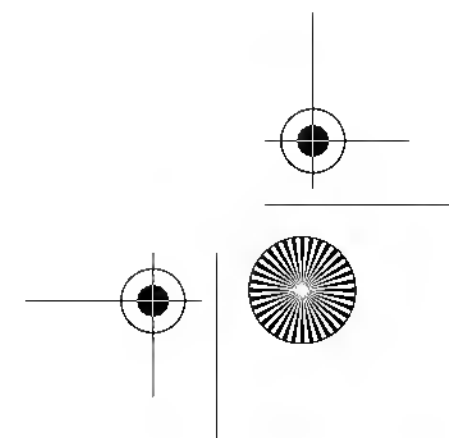
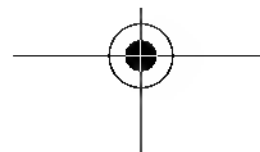
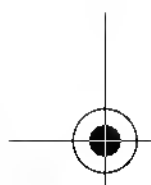
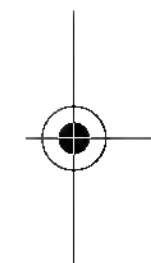
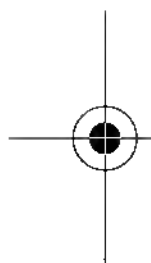
3.4.4.2 Inoculum Size

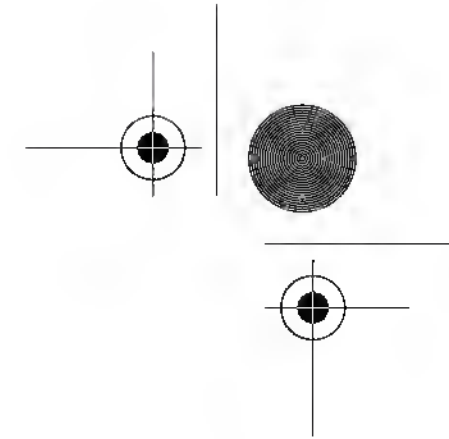
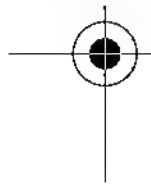
Masana and Baranyi (2000b) indicated that inoculum size affected the position of the boundary. Robinson et al. (1998) reported similar effects of inoculum density on bacterial lag times. While it is clear that time to detection would depend on inoculum density, growth detection methods were not cell-density-dependent in those studies. Parente et al. (1998) also reported that a decrease in inoculum size decreased the probability of survival. If the shock of transfer is known to inactivate a fixed proportion of the cells in the inoculum, to develop a robust model it will be necessary to use an inoculum that ensures that even after inactivation there is a high probability that at least one cell will survive.

The above observations lend support to the hypothesis that it is the distribution of physiological states of readiness to survive and multiply in a new environment that determines the position of the G/NG boundary, i.e., all other things being equal, the more cells in the inoculum the more likely it is that there is one cell that has the capacity to survive and grow. This also reinforces the equivalence between G/NG boundary modeling and the modeling of conditions that lead to infinite lag times. The importance of the distribution of lag times on the observed lag times of whole populations has been discussed by Baranyi (1998).

There may be more involved reasons for inoculum density-dependent responses also, such as chemical messaging between cells (see, e.g., Miller and Bassler, 2001; Winans and Bassler, 2000).

In conclusion, if the aim is to determine absolute limits to growth, a higher number of cells is preferable. Masana and Baranyi (2000b) stated that growth boundaries “represented the chance of growth for each sample; therefore, to assure a low probability of growth in many samples, it will be more relevant to consider





boundaries for high inoculum levels.” Equally, as noted above, steps to maximize the cell’s chances of survival and growth in the environment are also recommended.

There is potentially a caveat, however, that needs to be applied. Maximum population densities of batch cultures are reported to decline under increasingly harsh growth conditions. Thus, the use of high inocula may mask the true position of the G/NG boundary if the inoculum used is already denser than the MPD of the organism in a very stressful test environment.

3.4.4.3 Are There Absolute Limits to Microbial Growth?

In the above discussion it has been implicitly assumed that there are absolute limits to microbial growth under combined environmental stresses. It is pertinent to examine this assumption.

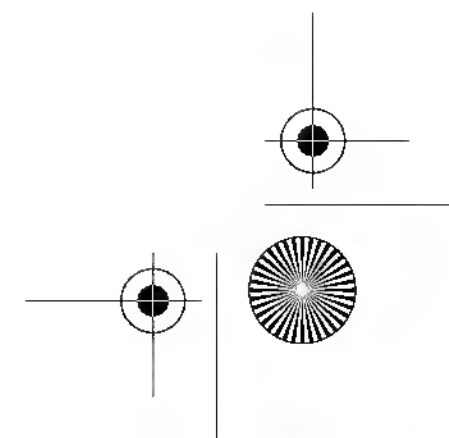
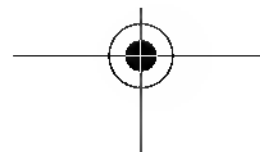
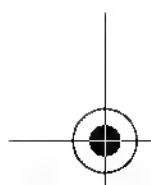
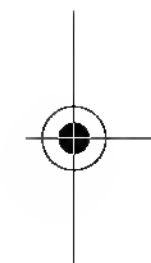
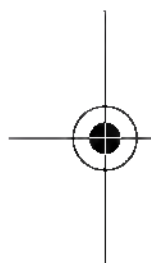
Numerous authors have noted that, within an experiment, the transition between conditions that allow growth, and those that do not, is abrupt and that usually all replicates at the last growth condition grow, while all the replicates at the first-growth-preventing condition do not (Masana and Baranyi, 2000; Presser et al., 1998; Tienungoon et al., 2000). McKellar and Xu (2001), for example, reported that of 1820 conditions tested, all live replicates of each condition either grew or did not grow. This abruptness, however, is not always evident in the modeled results (Tienungoon et al., 2000).

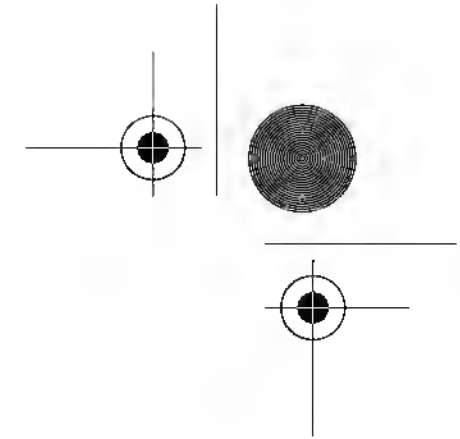
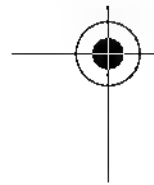
Conversely, between experiments by the same researcher, using the same methods and the same strain, results are not always reproducible. Figure 3.9 provides an example and Masana and Baranyi (2000b) make the same observation of their data for *Brochothrix thermosphacta*. At the same time, however, there is evidence of excellent reproducibility of boundaries between independent workers, using different strains, and different methods in different locations. The results of Tienungoon et al. (2000) were highly consistent between two strains tested, and more notably, with those of George et al. (1988) and Cole et al. (1990) presented a decade earlier, including different strains in one case. There is also a remarkable similarity between the pH/temperature G/NG interface of *Listeria innocua* reported by Le Marc et al. (2002) and the same interface for two species of *L. monocytogenes* presented in Tienungoon et al. (2000).

Jenkins et al. (2000) noted that the boundary they derived for the growth limits of *Zygosaccharomyces baillii* in beverages was very consistent with a model developed 30 years earlier for the stability of acidic sauces.

Stewart et al. (2002) noted that with *S. aureus*, as conditions became increasingly unfavorable for growth, the contour lines (P_{growth}) they generated drew closer and closer together, suggesting that conditions were approaching absolute limits that do not allow growth. Conversely, there are examples where one group’s observations do not agree well with another’s for an analogous organisms/environmental pair (e.g., Bolton and Frank, 1999; McKellar and Xu, 2001). Delignette-Muller and Rosso (2000) reported strain variability in the minimum temperature for growth.

While the above discussion points to heterogeneity in the physiological readiness of bacteria to grow in a new environment, Masana and Baranyi (2000b) also infer that differences in microenvironments, particularly within foods, could also be a source of heterogeneity in observed growth limits.





In conclusion, there is a body of experimental evidence that suggests that growth boundaries, if carefully determined, might be highly reproducible. Conversely, counterexamples exist. It remains to be determined whether the incongruous results arise from significant and measurable differences in methodology, e.g., the detection time used in the respective studies, or are due to uncontrollable sources (Table 3.7).

3.4.4.4 Experimental Design

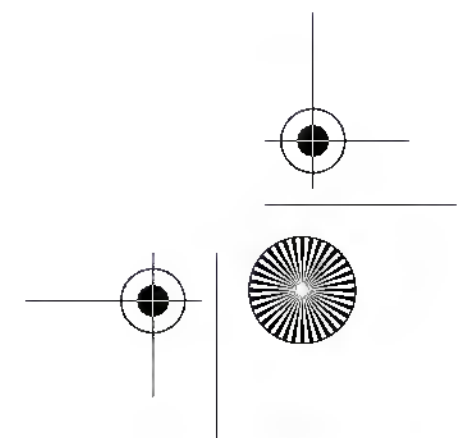
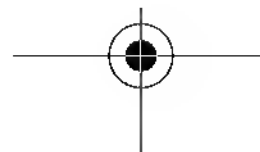
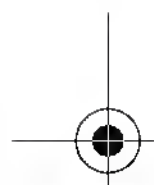
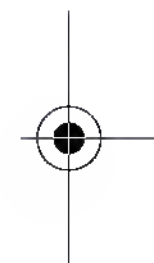
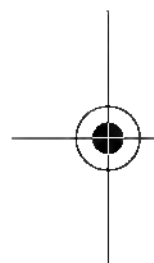
As noted above, it is not possible from first principles to design the optimal experiment that captures the information to characterize the G/NG boundary. Various authors have suggested physiological interpretations (Battey et al., 2001; Battey and Schaffner, 2001; Jenkins et al., 2000; López-Malo et al., 2000; McMeekin et al., 2000) but none have yet been experimentally tested.

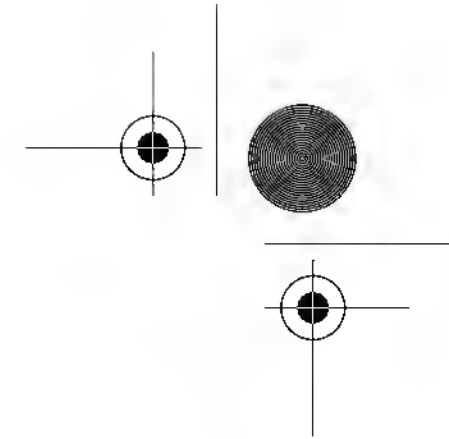
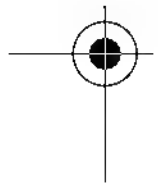
Thus, an empirical approach that aims to collect as much information in the region of most interest, i.e., the G/NG interface, is recommended by most workers. Several groups of researchers have indicated that they use a two-stage modeling process. The first uses a coarse grid of conditions of variables to roughly establish the position of the boundary. The second phase monitors responses at conditions near the boundary and at close intervals of the environmental parameters. Variable combinations far from the interface, at which growth is either highly likely or highly unlikely, do not provide much information to the modeling process, which seeks to define the interface with a high level of precision. Equally, it is ideal to use a design that gives roughly equal numbers of conditions where growth is, and growth is not, observed (Jenkins et al., 2000; Legan et al., 2002; Masana and Baranyi, 2000b; Uljas et al., 2001). Pragmatically, Legan et al. (2002) recommend setting up “marginal” and “no-growth” treatments first because these treatments will run for the longest time (possibly several months). Those conditions in which growth is expected to be relatively quick can be set up last because they only need monitoring until growth is detected.

The nature of these studies necessarily involves long incubation times. Legan et al. (2002) noted that particular care must be taken to ensure that the initial conditions do not change over time solely as a result of an uncontrolled interaction with the laboratory environment. Prevention of desiccation or uptake of water vapor requires particular attention. Changes resulting from microbial activity may, however, be an important part of the mechanism leading to growth initiation and should not be stabilized at the expense of growth that would naturally occur in a food. Legan et al. (2002) comment that, for example, maintaining the initial pH over time is typically neither possible nor practical, even in buffered media, and that allowing a change in pH due to growth of the organism more closely mimics what would happen in a food product than maintaining the initial pH over time.

3.4.4.5 Conclusion

From the above discussion, unambiguous definition of the G/NG boundary of an organism in multidimensional space presents several paradoxical challenges. While an experimenter will do well to remember these considerations in the interpretation





of his/her results, it seems probable that methods that have been used to date will have come close to identifying the “true” G/NG boundary, and that the position of the boundary will move only slightly if an experimenter acts to control all of the above variables and to maximize the potential for the observation of growth in the chosen experimental system.

While the discussion has not focused specifically on appropriate methods for probability of growth within a defined time, many of the same principles and considerations will apply.

Moreover, the field of growth limits modeling, while having an equally long history as kinetic modeling, now seems to be quite disjointed, with little rigorous comparison of approaches, let alone agreement on the most appropriate model structures or experimental methods. In particular, the earlier work in probability modeling seems to have been ignored by some more contemporary workers, without reasons being indicated.

The results of G/NG studies are clearly of great interest to food producers and food safety managers. It is perhaps time, then, that the G/NG modeling community seeks to find common ground and to begin to develop a rigorous framework for the development, and interpretation, of growth limits studies.

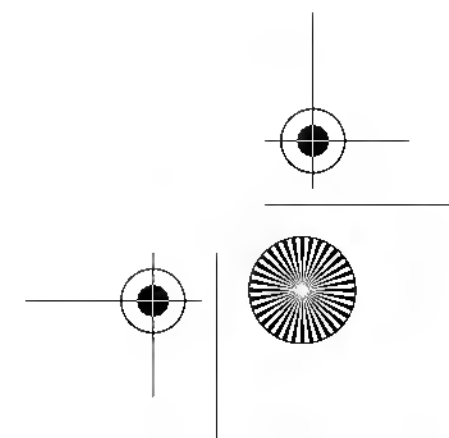
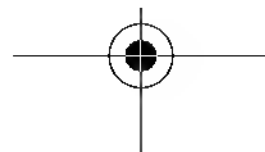
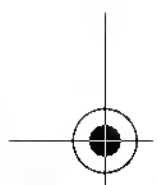
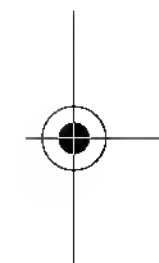
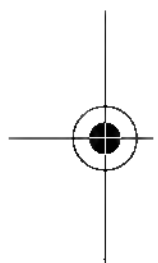
APPENDIX A3.1 — CHARACTERIZATION OF ENVIRONMENTAL PARAMETERS AFFECTING MICROBIAL KINETICS IN FOODS

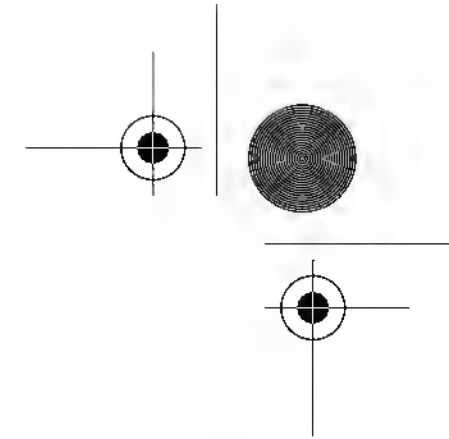
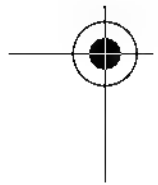
A3.1.1 TEMPERATURE

In most situations, temperature is the major environmental parameter influencing kinetics of microorganisms in food and its effect is included in most predictive microbiology models. During processing, storage, and distribution the temperature of foods can vary substantially, frequently including periods of temperature abuse for chilled foods (see, e.g., Audits International, 1999; James and Evans, 1990; O’Brien, 1997; Sergelidis et al., 1997). Thus, it is an important property of secondary models that they can predict the effect of changing temperatures on microbial kinetics and application of these models relies on information about product temperature and its possible variation over time. Numerous types of thermometers, temperature probes, and data loggers are available (McMeekin et al., 1993, pp. 257–269; seagrant.oregonstate.edu/extension/sheng/loggers.html) to measure the temperature of foods or food processing equipment. Infrared non-contact thermometers are often appropriate for foods but their use is limited for process equipment with stainless surfaces.

A3.1.2 STORAGE ATMOSPHERE

Foods are typically stored aerobically, vacuum packed, or by using modified atmosphere packing (MAP). “Controlled atmosphere packaging” can be considered a special case of MAP. MAP foods are exposed to an atmosphere different from both





air and vacuum packed usually involving mixtures of the gasses carbon dioxide (CO₂), nitrogen (N₂), and oxygen (O₂).

O₂ and CO₂ influence growth of most microorganisms and secondary predictive models must take their effect into account. The solubility of O₂ in water, and thereby into the water phase of foods, is low (~0.03 l/l) but it can be important for growth and metabolism of microorganisms in both aerobic and MAP-stored products (Dainty and Mackey, 1992). Numerous techniques and instruments are available to determine O₂ in the gas phase or dissolved in food. Microelectrodes to determine gradients of dissolved O₂ in foods are available (www.instechlabs.com/oxygen.html; www.microelectrodes.com/) but models to predict the effect of such gradients remain to be developed. To account for the effect of aerobic or vacuum packed storage of foods a categorical approach has been used within predictive microbiology. For aerobic conditions growth media with access to air have been agitated. For vacuum packed foods microorganisms typically have been grown under 100% N₂.

CO₂ inhibits growth of some microorganisms substantially and, to predict microbial growth in MAP foods, it is important to determine the equilibrium concentration in the gas phase or the concentration of CO₂ dissolved into the foods water phase. At equilibrium, the concentration of CO₂ dissolved into the water phase of foods is proportional to the partial pressure of CO₂ in the atmosphere surrounding the product. Henry's law (Equation A3.1) provides a good approximation for the solubility of CO₂.

$$[CO_2]_{aqueous}^{Equilibrium} = K_H \cdot \rho CO_2 \quad (A3.1)$$

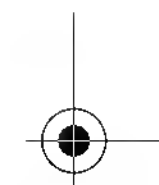
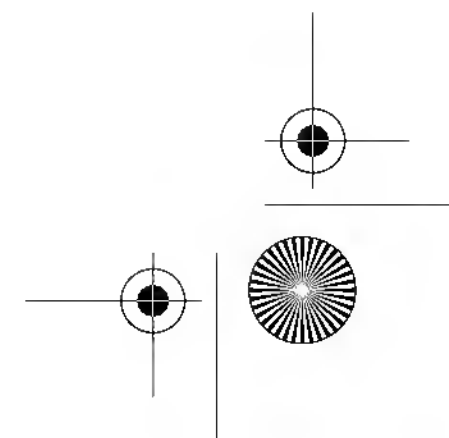
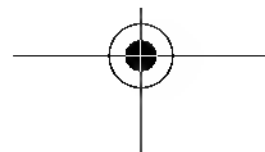
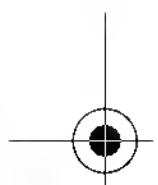
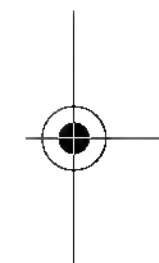
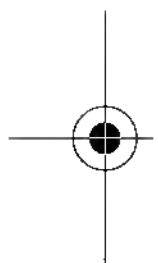
In Equation A3.1, K_H is Henry's constant (mg/l/atm) and ρCO_2 is the partial pressure (atm) of CO₂. Between 0 and 160°C the temperature dependence of the Henry's constant can be predicted by Equation A3.2:

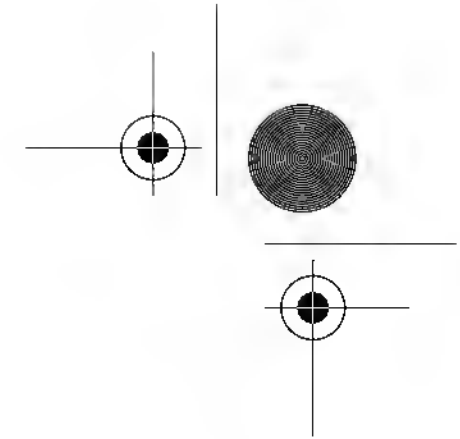
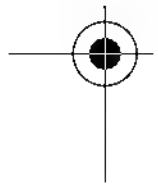
$$K_H (mg \cdot l^{-1} \cdot atm^{-1}) = \frac{101325 \cdot 2.4429}{\exp(-6.8346 + 1.2817 \cdot 10^4 / K - 3.7668 \cdot 10^6 / K^2 + 2.997 \cdot 10^3 / K^3)} \quad (A3.2)$$

where K is the absolute temperature (Carroll et al., 1991). Those authors expressed K_H as MPa/mole fraction. In Equation A3.2 the constants 101,325 Pa/atm and 2.4429 was used to convert this unit into mg CO₂/l H₂O/atm.

For MAP foods in flexible packaging the partial pressure of CO₂ is conveniently determined from the percentage of CO₂ inside the pack. A range of analytical methods is available to determine CO₂ concentration in gas mixtures or concentrations of dissolved CO₂ (Dixon and Kell, 1989; www.pbi-dansensor.com/Food.htm).

As shown from Equation A3.1 and Equation A3.2, the concentration of CO₂ dissolved in the water phase of a MAP food with 50% CO₂ in the headspace gas at equilibrium is 1.67 g/l at 0°C and 1.26 g/l at 8°C. Because of the high solubility of





CO₂ in water the gas composition in the headspace of MAP foods changes after packaging. The equilibrium gas composition is influenced by several factors, e.g., the percentage of CO₂ in the initial headspace gas (%CO₂^{Initial}), the initial gas/product volume ratio (G/P), temperature, pH, lipids in the food, respiration of the food, and, of course, permeability of the packaging film. Different mass-balance equations to predict the rate of adsorption and solubility of CO₂ have been suggested (Devlieghere et al., 1998; Dixon and Kell, 1989; Gill, 1988; Löwenadler and Rönner, 1994; Simpson et al., 2001a,b; Zhao et al., 1995). In chilled foods the rate of absorption of CO₂ is rapid compared to growth of microorganisms. Therefore, to predict microbial growth in these MAP foods it is sufficient to take into account the equilibrium concentration of CO₂.

Devlieghere et al. (1998) suggested Equation A3.3 to predict the concentration of CO₂ in the water phase as a function of %CO₂^{Initial} and G/P. In Equation A3.3, d_{CO_2} is the density of CO₂ (1.976 g/l).

$$[CO_2]_{aqueous}^{Equilibrium} = \frac{\left(\frac{G}{P} \cdot d_{CO_2} + K_H\right) - \sqrt{\left(\frac{G}{P} \cdot d_{CO_2} + K_H\right)^2 - \left(\frac{4}{100} \cdot K_H \cdot \frac{G}{P} \cdot \%CO_2^{Initial} \cdot d_{CO_2}\right)}}{2} \quad (A3.3)$$

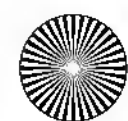
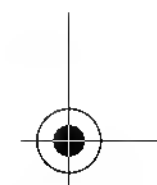
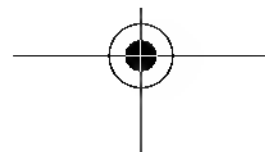
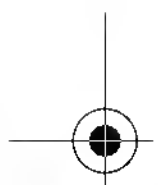
Equation A3.3 does not take into account the effect of the storage temperature and Devlieghere et al. (1998) developed a polynomial model to predict the concentration of dissolved CO₂ as a function of %CO₂^{Initial}, G/P, and temperature. If, for example, %CO₂^{Initial} is 25, the polynomial model predicts that a G/P ratio of three results in higher concentration of dissolved CO₂ than does a G/P ratio of 4, which is not logical. In contrast we have found that the combined use of Equation A3.2 and Equation A3.3 provides realistic predictions for concentrations of dissolved CO₂. It also seems relevant to include the effect of product pH on dissolved CO₂, and thereby the equilibrium concentration of CO₂ in the gas phase of MAP foods.

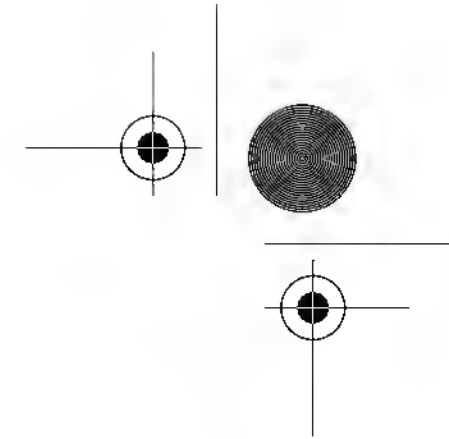
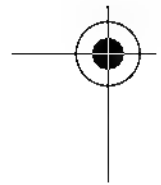
A3.1.3 SALT, WATER-PHASE SALT, AND WATER ACTIVITY

While temperature is the single most important *storage* condition influencing growth of microorganisms in foods, NaCl is the most important *product* characteristic in many foods. The concentration of NaCl in foods can be determined as chloride by titration (Anon., 1995a). Instruments to determine NaCl indirectly from conductivity measurements are available but extensive calibration for particular types of products may be required. In fresh and intermediate moisture foods, NaCl is dissolved in the water phase of the products.

To predict the effect of NaCl on growth of microorganisms in these products the concentration of water-phase salt (WPS) or relative humidity, i.e., the water activity (a_w) must be determined (Equation A3.4 to Equation A3.7).

Water-phase salt can be calculated from Equation A3.4:





$$\% \text{ Water phase salt} = \frac{\% \text{NaCl (w/v)} \times 100}{100 - \% \text{ dry matter} + \% \text{NaCl (w/v)}} \quad (\text{A3.4})$$

Water activity is a fundamental property of aqueous solutions and is defined as:

$$a_w = \frac{\rho}{\rho_o} \quad (\text{A3.5})$$

where ρ is the vapor pressure of the solution and ρ_o is the vapor pressure of the pure water under the same conditions of temperature, etc.

For mixtures of NaCl and water there is a direct relation between the WPS content and a_w (Resnik and Chirife, 1988; Equation A3.6 and Equation A3.7). For cured foods where NaCl is the only major humectant these relations are valid as documented, e.g., for cold-smoked salmon (Jørgensen et al., 2000) and processed “delicatessen” meats (Ross and Shadbolt, 2001). To determine water activity of foods, instruments relying on the dew point method are now widely used because of their speed (providing results within a few minutes), robustness, and reliability but other methods and instruments are available (Mathlouthi, 2001).

$$a_w = 1 - 0.0052471 \cdot \%WPS - 0.00012206 \cdot \%WPS^2 \quad (\text{A3.6})$$

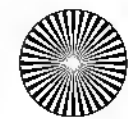
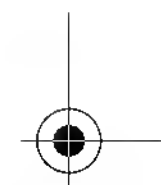
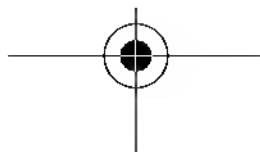
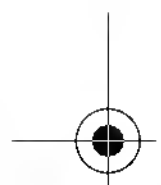
$$\%WPS = 8 - 140.07 \cdot (a_w - 0.95) - 405.12 \cdot (a_w - 0.95)^2 \quad (\text{A3.7})$$

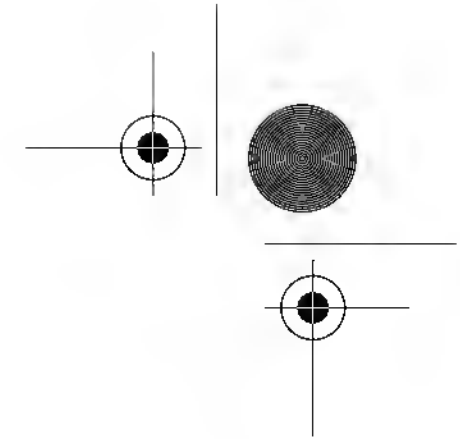
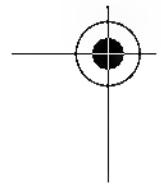
A3.1.4 pH

For many microorganisms, small pH variations in the pH range ~6 to ~7 have very little or no effect on population kinetics. In more acidic foods, however, pH *per se* can greatly influence microbial kinetics but can also accentuate the effect of other added preservative compounds. The pH of solid foods is often determined by homogenizing 10 g of a sample with 10 to 20 ml of distilled water and measuring the pH of the suspension using a standard combined electrode.

A3.1.5 ADDED PRESERVATIVES INCLUDING ORGANIC ACIDS, NITRATE, AND SPICES

High concentrations of organic acids occur naturally in some foods and various organic acids including acetic acid, ascorbic acid, benzoic acid, citric acid, lactic acid, and sorbic acid are frequently added to foods. Organic acids can inhibit growth of microorganisms markedly and secondary models to predict their inhibitory effect are frequently needed. As for NaCl the secondary models must take into account the concentration of organic acids in the water phase of products. In addition, secondary models may need to describe the combined effect of organic acids and other environmental parameters particularly the pH.





In solution, organic acids exist either as the dissociated (ionized) or undissociated species. The Henderson–Hasselbalch equation (Equation A3.8) relates the proportion of undissociated and dissociated forms of organic acid to pH and pK_a according to the following expression:

$$[A^-]/[HA] = 10^{pH-pK_a} \quad (A3.8)$$

where [HA] is the concentration of undissociated form of the acid, $[A^-]$ the concentration of dissociated (ionized) form of the acid, and pK_a is the pH at which the concentrations of the two forms are equal.

While both the dissociated and the undissociated forms of organic acids have inhibitory effects on bacterial growth the undissociated form is more inhibitory, usually by two to three orders of magnitude, than the dissociated form (Eklund, 1989).

Cross-multiplying and rearranging Equation A3.8 to solve for [HA] gives:

$$[HA] = [LAC]/(1 + 10^{pH-pK_a}) \quad (A3.9)$$

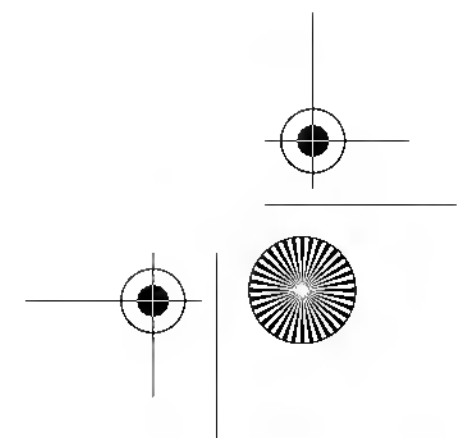
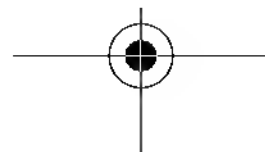
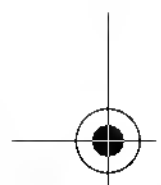
where [LAC] is the total lactic acid concentration and all other terms are as previously defined.

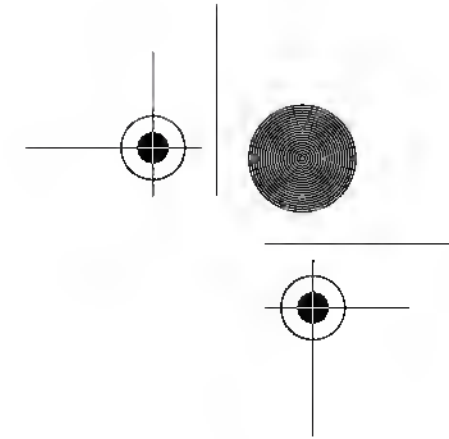
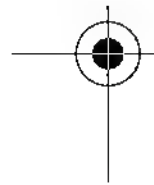
As the concentration of an undissociated acid increases the growth rate of microorganisms decreases, eventually ceasing completely at a level described as the MIC. This behavior, and its dependence on the interaction of pH and total organic acid concentration, is included explicitly in several secondary models (Augustin and Carrier, 2000a; Presser et al., 1997).

Simple enzyme kits are available to determine several of the organic acids that are important in foods. Simultaneous determination of a range of organic acids is possible by HPLC analysis and is often an appropriate method to use (Dalgaard and Jørgensen, 2000; Pecina et al., 1984).

Nitrite can be added to some types of meat products and its concentration in the water phase of products must be taken into account when secondary predictive models for these products are developed. Colorimetric methods are available to measure the concentration of nitrite in foods (Anon., 1995b; Karl, 1992).

Spices and herbs can have substantial antimicrobial activity and appropriate terms may need to be included in secondary models (Koutsoumanis et al., 1999; Skandamis and Nychas, 2000). The concentration of active antimicrobial components in spices, herbs, and essential oils can vary substantially as a function, e.g., of geographical region and season (Nychas and Tassou, 2000; Sofos et al., 1998). Therefore, the development of accurate secondary predictive models most likely will have to rely on the concentration of their active antimicrobial components. Recently, Lambert et al. (2001) showed the antimicrobial effect of the oregano essential oil quantitatively corresponded to the effect of its two active components, i.e., thymol and carvacrol. To quantitatively determine active components in spices, herbs, and essential oils appropriate extracts can be analyzed by GC/MS techniques (Cosentino et al., 1999; Cowan, 1999).





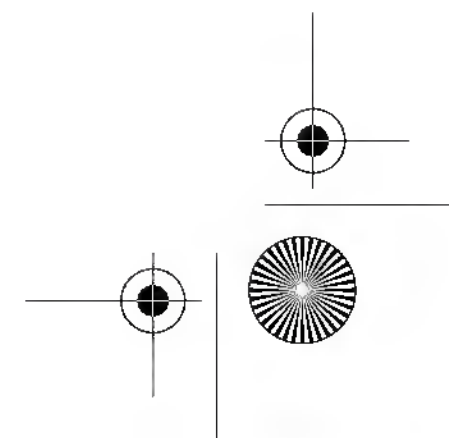
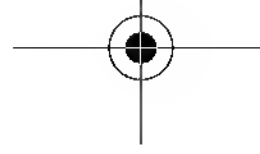
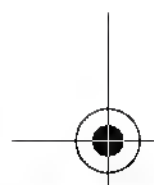
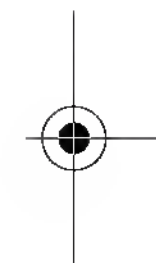
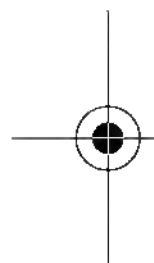
A3.1.6 SMOKE COMPONENTS

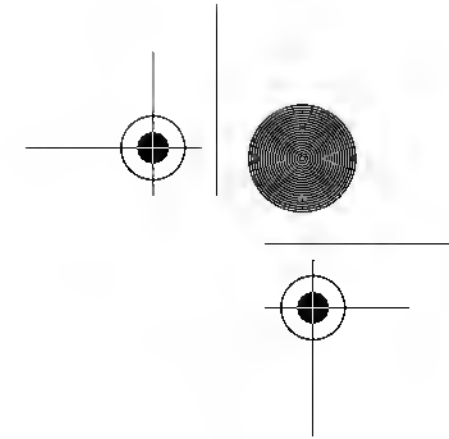
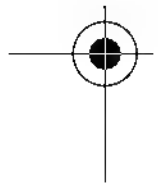
It has long been known that high concentrations of smoke components have strong antimicrobial activity (Shewan, 1949). Today many meat and seafood products are smoked but typically less intensively than some decades ago. However, even moderate concentrations of smoke components can influence growth rates, growth limits, and rates of death/inactivation of microorganisms in foods (Leroi et al., 2000; Leroi and Joffraud, 2000; Ross et al., 2000b; Suñen, 1998; Thurette et al., 1998). Thus, to obtain accurate prediction of microbial kinetics in smoked foods it is important to include terms for the effect of smoke components in secondary models. Phenols are important antimicrobials in wood smoke, or in liquid smokes, and a few secondary models include the total phenol concentration as an environmental parameter (Augustin and Carlier 2000a,b; Giménez and Dalgaard, in press; Membré et al., 1997).

Classical colorimetric methods can be used to determine the total concentration of phenols in smoked foods. These methods rely on formation of colored complexes, e.g., between phenols and Gibb's reagent (2,6-dichloroquinone-4-chloroimide) or 4-aminoantipyrine (Leroi et al., 1998; Tucker, 1942). The total phenol concentration is a crude measure of how intensely foods have been smoked. By using GC/MS techniques more detailed information about specific smoke components can be obtained (Guillén and Errecalde, 2002; McGill et al., 1985; Tóth and Potthast, 1984). In the future, secondary models may be developed to include the effect of specific phenols, other specific smoke components, and possibly their interaction with NaCl. During the smoking of foods, phenols and other smoke components are mainly deposited in the outer 0.5 cm of the product (Chan et al., 1975). Modeling the effect of the spatial distribution in foods is another challenge.

A3.1.7 OTHER ENVIRONMENTAL PARAMETERS

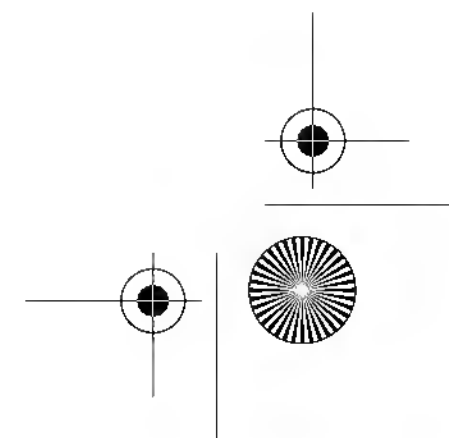
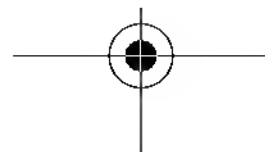
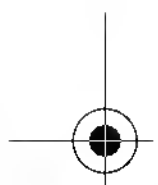
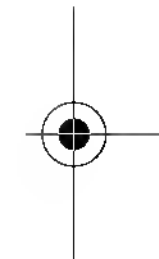
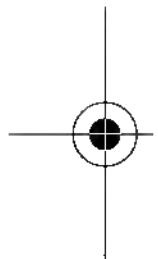
The environmental parameters discussed above include those that are of major importance in traditional methods of food preservation. Many modern methods of food preservation also rely on combinations of these environmental parameters. However, the effect of a few well-known and several emerging food processing technologies relies on the antimicrobial effect of other environmental parameters, e.g., bacteriocins, gamma irradiation, high electric field pulses, high pressure, and UV light. Secondary models for the effect of some of these environmental parameters have been developed but will not be discussed here in detail. Other environmental parameters related to food structure and to the effect of microbial metabolism on changes in environmental parameters are discussed in [Chapter 5](#) whereas the effect of time-varying environmental parameters is discussed in [Chapter 7](#).

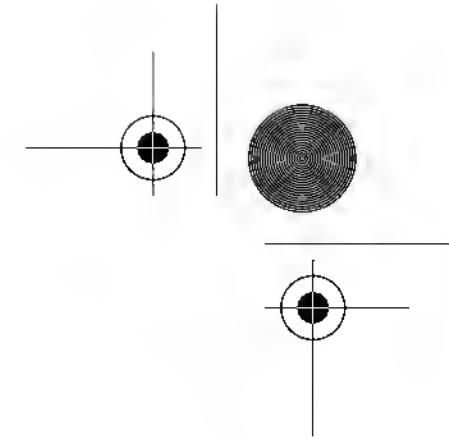
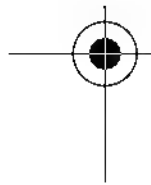




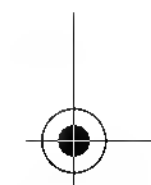
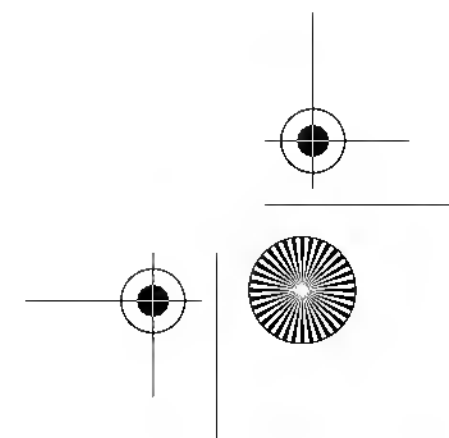
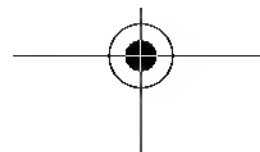
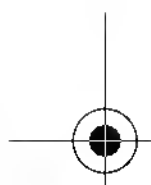
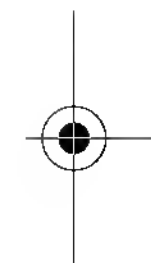
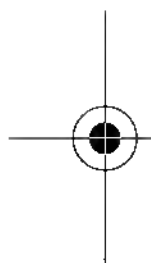
REFERENCES

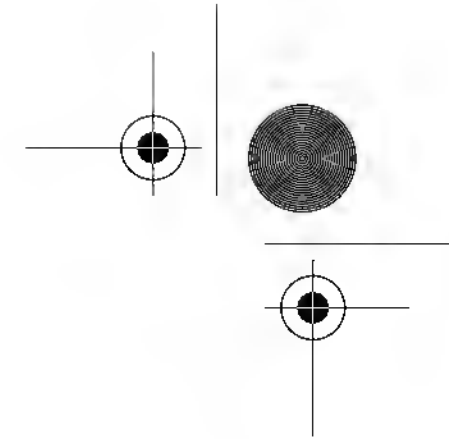
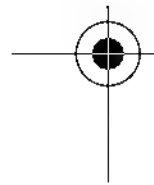
- Abdullah, B., Gardini, F., Paparella, A., and Guerzoni, M.E. Growth modelling of the predominant microbial groups in hamburgers in relation to modulation of atmosphere composition, storage temperature, and diameter of meat particle. *Meat Sci.*, 38, 511–526, 1994.
- Adair, C., Kilsby, D.C., and Whittall, P.T. Comparison of the Schoolfield (non-linear Arrhenius) model and the square root model for predicting bacterial growth in foods. *Food Microbiol.*, 6, 7–18, 1989.
- Adams, M.R., Little, C.L., and Easter, M.C. Modelling the effect of pH, acidulant and temperature on growth of *Yersinia enterocolitica*. *J. Appl. Bacteriol.*, 71, 65–71, 1991.
- Anon. Salt (chlorine as sodium chloride) in seafood. Volumetric method. In *Official Methods of Analysis*, AOAC, Arlington, VA, 1995a, p. 937.09.
- Anon. Nitrites in cured meat. Colorimetric method. In *Official Methods of Analysis*, AOAC, Arlington, VA, 1995b, p. 937.31.
- Audits International, US Food Temperature Evaluation. Audits International, Northbrook, IL, 1999. http://www.foodriskclearinghouse.umd.edu/audits_international.htm. February, 2002.
- Augustin, J.-C. and Carlier, V. Mathematical modelling of the growth rate and lag time for *Listeria monocytogenes*. *Int. J. Food Microbiol.*, 56, 29–51, 2000a.
- Augustin, J.-C. and Carlier, V. Modelling the growth of *Listeria monocytogenes* with a multiplicative type model including interactions between environmental factors. *Int. J. Food Microbiol.*, 56, 53–70, 2000b.
- Augustin, J.-C., Rosso, L., and Carlier, V. A model describing the effect of temperature history on lag time for *Listeria monocytogenes*. *Int. J. Food Microbiol.*, 57, 169–181, 2000.
- Bajard, S., Rosso, L., Fardel, G., and Flandrois, J.P. The particular behaviour of *Listeria monocytogenes* under sub-optimal conditions. *Int. J. Food Microbiol.*, 29, 201–211, 1996.
- Baker, D.A. Probability models to assess the safety of foods with respect to *Clostridium botulinum*. *J. Ind. Microbiol.*, 12, 156–161, 1993.
- Baker, D.A. and Genigeorgis, C. Predicting the safe storage of fresh fish under modified atmospheres with respect to *Clostridium botulinum* toxigenesis by modelling length of the lag phase of growth. *J. Food Prot.*, 53, 131–140, 1990.
- Baker, D.A. and Genigeorgis, C. Predictive modeling. In *Clostridium botulinum Ecology and Control in Foods*, Eds. A.W.H. Hauschild and K.L. Dodds. Marcel Dekker, New York, 1993, 343–406.
- Baker, D., Genigeorgis, C., Glover, J., and Razavilar, V. Growth and toxigenesis of *C. botulinum* type E in fishes packaged under modified atmospheres. *Int. J. Food Microbiol.*, 10, 269–290, 1990.
- Baranyi, J. Comparison of stochastic and deterministic concepts of bacterial lag. *J. Theor. Biol.*, 192, 403–408, 1998.
- Baranyi, J. and Roberts, T.A. A dynamic approach to predicting bacterial growth in food. *Int. J. Food Microbiol.*, 23, 277–294, 1994.
- Baranyi, J. and Roberts, T.A. Mathematics of predictive food microbiology. *Int. J. Food Microbiol.*, 26, 199–218, 1995.
- Baranyi, J., Ross, T., McMeekin, T.A., and Roberts, T.A. Effects of parametrization on the performance of empirical models used in “predictive microbiology.” *Food Microbiol.*, 13, 83–91, 1996.



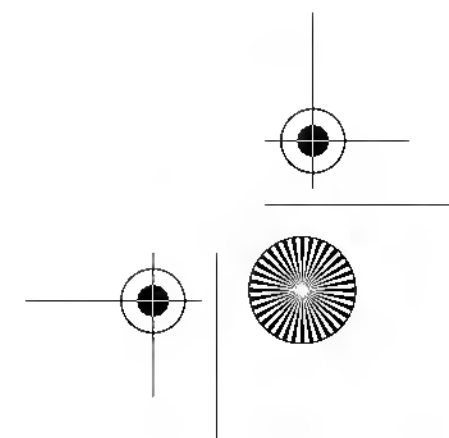
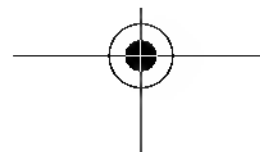
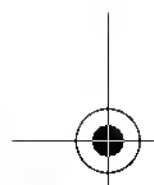
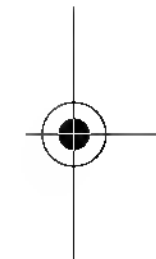
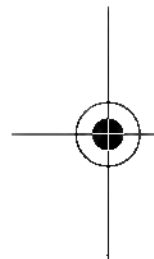


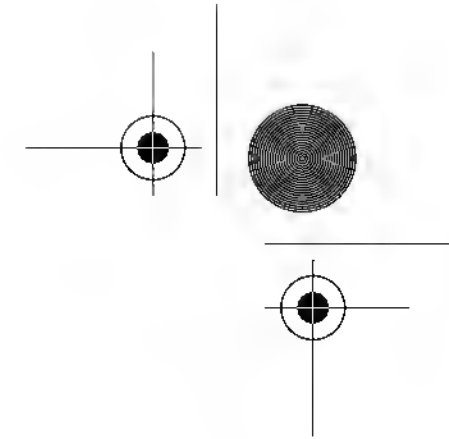
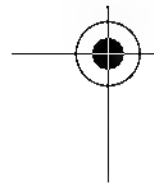
- Batthey, A.S. and Schaffner, D.W. Modelling bacterial spoilage in cold- lled ready to drink beverages by *Acinetobacter calcoaceticus* and *Gluconobacter oxydans*. *J. Appl. Microbiol.*, 91, 237–247, 2001.
- Batthey, A.S., Duffy, S., and Schaffner, D.W. Modelling mould spoilage on cold- lled ready-to-drink beverages by *Aspergillus niger* and *Penicillium spinulosum*. *Food Microbiol.*, 18, 521–529, 2001.
- Batthey, A.S., Duffy, S., and Schaffner, D.W. Modeling yeast spoilage in cold- lled ready-to-drink beverages with *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Candida lipolytica*. *Appl. Environ. Microbiol.*, 68, 1901–1906, 2002.
- Benedict, R.C., Pertridge, T., Wells, D., and Buchanan, R.L. *Bacillus cereus*: aerobic growth kinetics. *J. Food Prot.*, 56, 211–214, 1993.
- Bernaerts, K., Versyck, K.J., and van Impe, J.F. On the design of optimal experiments for parameter estimation of a Ratkowsky-type growth kinetic at suboptimal temperatures. *Int. J. Food Microbiol.*, 54, 27–38, 2000.
- Bhaduri, S., Buchanan, R.L., and Phillips, J.G. Expanded response surface model for predicting the effects of temperature, pH, sodium chloride contents and sodium nitrite concentration on the growth rate of *Yersinia enterocolitica*. *J. Appl. Bacteriol.*, 79, 163–170, 1995.
- Bolton, L.F. and Frank, J.F. De ning the growth/no growth interface for *Listeria monocytogenes* in Mexican-style cheese based on salt, pH and moisture content. *J. Food Prot.*, 62, 601–609, 1999.
- Bouttefroy, A., Mansour, M., Linder, M., and Milliere, J.B. Inhibitory combinations of nisin, sodium chloride, and pH on *Listeria monocytogenes* ATCC 15313 in broth by an experimental design approach. *Int. J. Food Microbiol.*, 54, 109–115, 2000.
- Broughall, J.M. and Brown, C. Hazard analysis applied to microbial growth in foods: development and application of three-dimensional models to predict bacterial growth. *Food Microbiol.*, 1, 13–22, 1984.
- Broughall, J.M., Anslow, P., and Kilsby, D.C. Hazard analysis applied to microbial growth in foods: development of mathematical models describing the effect of water activity. *J. Appl. Bacteriol.*, 55, 101–110, 1983.
- Buchanan, R.L. Developing and distributing user-friendly application software. *J. Ind. Microbiol.*, 12, 251–255, 1993a.
- Buchanan, R.L. Predictive food microbiology. *Trends Food Sci. Technol.*, 41, 6–11, 1993b.
- Buchanan, R.L. and Bagi, K. Expansion of response surface models for the growth of *Escherichia coli* O157:H7 to include sodium nitrite as a variable. *Int. J. Food Microbiol.*, 23, 317–332, 1994.
- Buchanan, R.L. and Phillips, J.G. Updated models for the effects of temperature, initial pH, NaCl, and NaNO₂ on aerobic and anaerobic growth of *Listeria monocytogenes*. *Quant. Microbiol.*, 2, 103–128, 2000.
- Buchanan, R.L., Smith, J.L., McColgan, C., Marmer, B.S., Golden, M., and Dell, B. Response surface models for the effects of temperature, pH, sodium chloride, and sodium nitrite on the aerobic and anaerobic growth of *Staphylococcus aureus* 196E. *J. Food Saf.*, 13, 159–175, 1993.
- Buchanan, R.L., Golden, M.H., and Phillips, J.G. Expanded models for the non-thermal inactivation of *Listeria monocytogenes*. *J. Appl. Microbiol.*, 82, 567–577, 1997.
- Budavari, S. *The Merck Index: An Encyclopaedia of Chemicals, Drugs, and Biologicals*, 11th ed., Merck and Co., Inc., Rahway, NJ, 1989.
- Carroll, J.J., Slupsky, J.D., and Mather, A.E. The solubility of carbon dioxide in water at low pressure. *J. Phys. Chem. Ref. Data*, 20, 1201–1209, 1991.



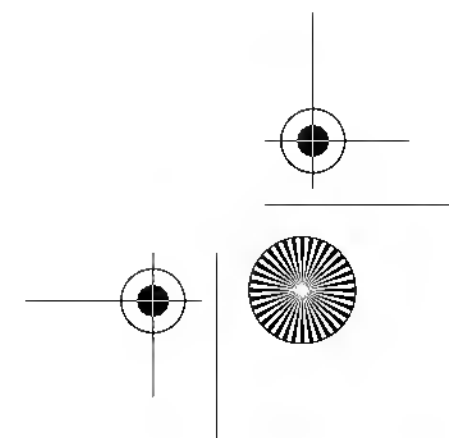
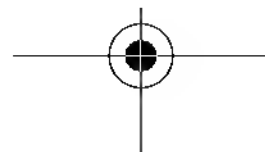
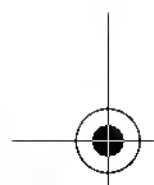
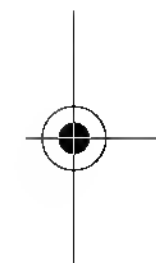
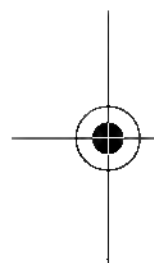


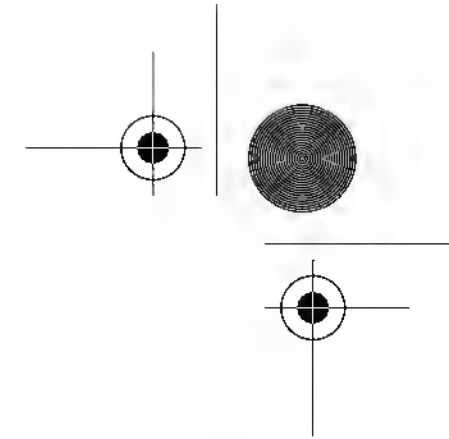
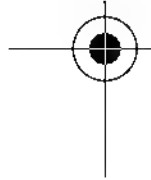
- Chan, W.S., Toledo, R.T., and Deng, J. Effect of smokehouse temperature, humidity and air flow on smoke penetration into fish muscle. *J. Food Sci.*, 40, 240–243, 1975.
- Chandler, R.E. and McMeekin, T.A. Combined effect of temperature and salt concentration/water activity on growth rate of *Halobacterium* spp. *J. Appl. Bacteriol.*, 67, 71–76, 1989.
- Chea, F.P., Chen, Y., Montville, T.J., and Schaffner, D.W. Modelling the germination kinetics of *Clostridium botulinum* 56A spores as affected by temperature, pH, and sodium chloride. *J. Food Prot.*, 63, 1071–1079, 2000.
- Cheroute-Vialette, M. and Lebert, A. Modelling the growth of *Listeria monocytogenes* in dynamic conditions. *Int. J. Food Microbiol.*, 55, 201–207, 2000.
- Chorin, E., Thuault, D., Cleret, J.-J., and Bourgeois, C.-M. Modelling *Bacillus cereus* growth. *Int. J. Food Microbiol.*, 38, 229–234, 1997.
- Cole, M.B., Jones, M.V., and Holyoak, C. The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. *J. Appl. Bacteriol.*, 69, 63–72, 1990.
- Connil, N., Plissoneau, L., Onno, B., Pilet, M.-F., Prévost, H., and Dousset, X. Growth of *Carnobacterium divergens* V41 and production of biogenic amines and divergicin in sterile cold-smoked salmon extract at varying temperatures, NaCl levels, and glucose concentrations. *J. Food Prot.*, 65, 333–338, 2002.
- Cosentino, S., Tuberoso, C.I.G., Pisano, B., Satta, M., Mascia, V., Arzedi, E., and Palmas, F. *In-vitro* antimicrobial activity and chemical composition of Sardinian *thymus* essential oils. *Lett. Appl. Microbiol.*, 29, 130–135, 1999.
- Cowan, M.M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12, 564–582, 1999.
- Cuppers, H.G.A., Oomes, S., and Brul, S. A model for the combined effects of temperature and salt concentration on growth rate of food spoilage molds. *Appl. Environ. Microbiol.*, 63, 3764–3769, 1997.
- Dainty, R.H. and Mackey, B.M. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *J. Appl. Bacteriol.*, 73, 103S–114S, 1992.
- Dalgaard, P. *Evaluation and Prediction of Microbial Fish Spoilage*, Ph.D. thesis, Technological Laboratory, Danish Ministry of Fisheries, Lynby, Denmark, 1993.
- Dalgaard, P. Modelling of microbial activity and prediction of shelf life for packed fresh fish. *Int. J. Food Microbiol.*, 26, 305–317, 1995.
- Dalgaard, P. Modelling and prediction the shelf-life of seafood. In *Safety and Quality Issues in Fish Processing*, Bremner, H.A. (Ed.), Woodhead Publishing, Cambridge, England, 2002, pp. 191–219.
- Dalgaard, P. and Jørgensen, L.V. Cooked and brined shrimps packed in a modified atmosphere have a shelf-life of >7 months at 0°C, but spoil at 4–6 days at 25°C. *Int. J. Food Sci. Technol.*, 35, 431–442, 2000.
- Dalgaard, P., Ross, T., Kamperman, L., Neumeyer, K., and McMeekin, T.A. Estimation of bacterial growth rates from turbidimetric and viable count data. *Int. J. Food Microbiol.*, 23, 391–404, 1994.
- Dalgaard, P., Mejlholm, O., and Huss, H.H. Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. *Int. J. Food Microbiol.*, 38, 169–179, 1997.
- Dalgaard, P., Cowan, B. J., Heilmann, J., and Silberg, S. The seafood spoilage and safety predictor (SSSP). In *Predictive Modeling in Foods — Conference Proceedings*, Van Impe, J.F.M., Geeraerd, A.H., Leguerinel, I., and Mafart, P. (Eds.), Katholieke Universiteit Leuven/BioTec, Belgium, 2003, pp. 256–258.



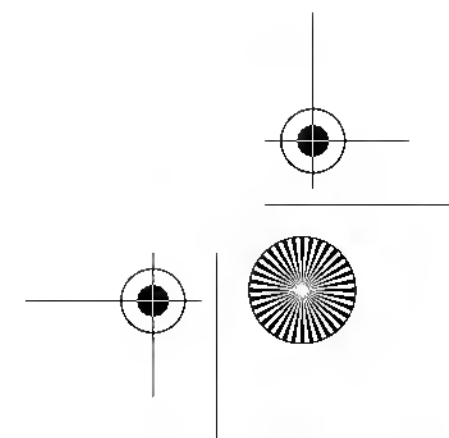
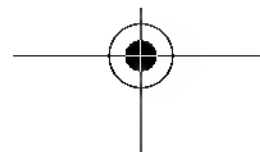
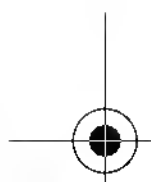
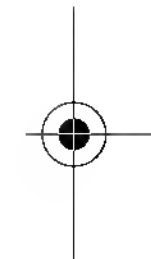
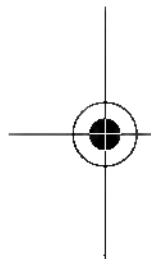


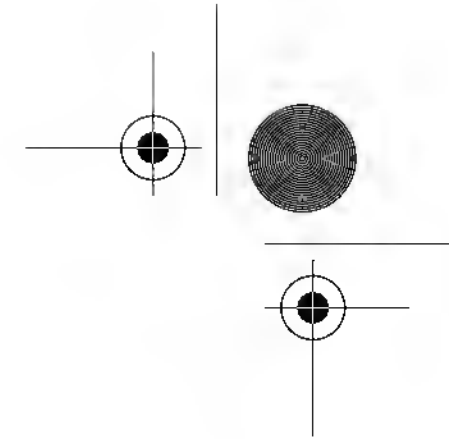
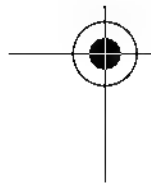
- Dantigny, P. Dimensionless analysis of the microbial growth rate dependence on sub-optimal temperatures. *J. Ind. Microbiol. Biotechnol.*, 21, 215–218, 1998.
- Dantigny, P. and Molin, P. Influence of the modelling approach on the estimation of the minimum temperature for growth in Belehradek-type models. *Food Microbiol.*, 17, 597–604, 2000.
- Daughtry, B.J., Davey, K.R., and King, K.D. Temperature dependence of growth kinetics of food bacteria. *Food Microbiol.*, 14, 21–30, 1997.
- Davey, K.R. A predictive model for combined temperature and water activity on microbial growth during the growth phase. *J. Appl. Bacteriol.*, 67, 483–488, 1989.
- Davey, K.R. Applicability of the Davey (linear Arrhenius) predictive model to the lag phase of microbial growth. *J. Appl. Bacteriol.*, 70, 253–257, 1991.
- Davey, K.R. Modeling the combined effect of temperature and pH on the rate coefficient for bacterial growth. *Int. J. Food Microbiol.*, 23, 295–303, 1994.
- Davey, K.R. Belehradek models: application to chilled foods preservation. *Refrig. Sci. Technol. Proc.*, EUR 18816, 37–47, 1999.
- Davey, K.R. Models for predicting the combined effect of environmental process factors on the exponential and lag phases of bacterial growth: development and application and an unexpected correlation. In *Proceedings of the 6th World Conference of Chemical Engineering*, Melbourne, 2001.
- Davey, K.R. and Daughtry, B.J. Validation of a model for predicting the combined effect of 3 environmental-factors on both exponential and lag phases of bacterial-growth — temperature, salt concentration and pH. *Food Res. Int.*, 28, 233–237, 1995.
- Davies, K.W. Design of experiments for predictive microbial modeling. *J. Ind. Microbiol.*, 12, 295–300, 1993.
- Deak, T. and Beuchat, L.R. Use of indirect conductimetry to predict the growth of spoilage yeasts, with special consideration of *Zygosaccharomyces bailii*. *Int. J. Food Microbiol.*, 23, 405–417, 1994.
- Delignette-Muller, M.L. Relation between the generation time and the lag time of bacterial growth kinetics. *Int. J. Food Microbiol.*, 43, 97–104, 1998.
- Delignette-Muller, M.L. and Rosso, L. Biological variability and exposure assessment. *Int. J. Food Microbiol.*, 58, 203–212, 2000.
- Delignette-Muller, M.L., Baty, F., Cornu, M., and Bergis, H. The effect of the temperature shifts on the lag phase of *Listeria monocytogenes*. In *Predictive Modeling in Foods—Conference Proceedings*, Van Impe, J.F.M., Geeraerd, A.H., Leguerinel, I., and Mafart, P. (Eds.), Katholieke Universiteit Leuven/BioTec, Belgium, 2003, pp. 209–211.
- Dengremont, E. and Membré, J.M. Statistical approach for comparison of the growth rates of the strains of *Staphylococcus aureus*. *Appl. Environ. Microbiol.*, 61, 4389–4395, 1997.
- Devlieghere, F., Debevere, J., and van Impe, J. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *Int. J. Food Microbiol.*, 43, 105–113, 1998.
- Devlieghere, F., Geeraerd, A.H., Versyck, K.J., Bernaert, H., van Impe, J., and Debevere, J. Shelf life of modified atmosphere packed cooked meat products: addition of N-lactate as a fourth shelf life determinative factor in a model and product validation. *Int. J. Food Microbiol.*, 58, 93–106, 2000a.
- Devlieghere, F., Lefevre, I., Magnin, A., and Debevere, J. Growth of *Aeromonas hydrophila* in modified-atmosphere-packed cooked meat products. *Food Microbiol.*, 17, 185–196, 2000b.



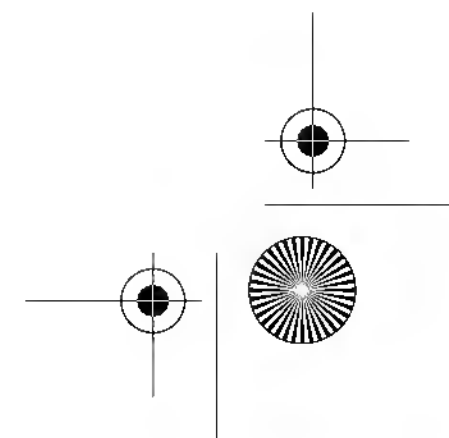
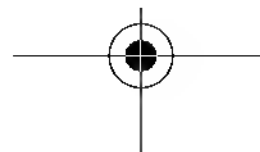
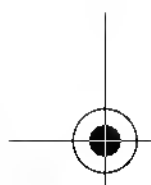
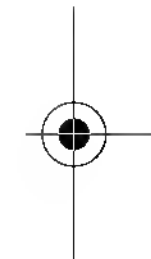
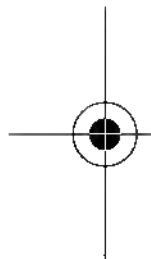


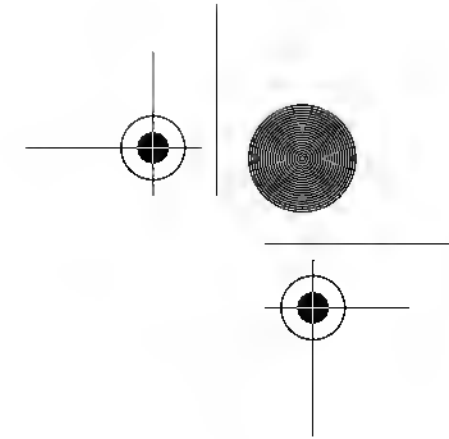
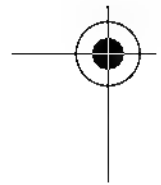
- Devlieghere, F., Geeraerd, A.H., Versyck, K.J., Vandewaetere, B., van Impe, J., and Debevere, J. Growth of *Listeria monocytogenes* in modified atmosphere packed cooked meat products: a predictive model. *Food Microbiol.*, 18, 53–66, 2001.
- Dixon, N.M. and Kell, D.B. The control and measurement of “CO₂” during fermentations. *J. Microbiol. Methods*, 10, 155–176, 1989.
- Dodds, K.L. An introduction to predictive microbiology and the development and use of probability models with *Clostridium botulinum*. *J. Ind. Microbiol.*, 12, 139–143, 1993.
- Doe, P.E. and Heruwati, E. A model for the prediction of the microbial spoilage of sun-dried tropical fish. *J. Food Eng.*, 8, 47–72, 1988.
- Draper, N.R. and Smith, H. *Applied Regression Analysis*, 2nd ed., John Wiley & Sons, New York, 1981, 709 pp.
- Eifert, J.D., Hackney, C.R., Pierson, M.D., Duncan, S.E., and Eigel, W.N. Acetic, lactic, and hydrochloric acid effects on *Staphylococcus aureus* 196E growth based on a predictive model. *J. Food Sci.*, 62, 174–178, 1997.
- Eklund, T. Organic acids and esters. In *Mechanisms of Action of Food Preservation Procedures*, Gould, G.W. (Ed.), Elsevier Science Publishers, London, 1989, pp. 160–200.
- Eyring, H. The activated complex in chemical reactions. *J. Chem. Phys.*, 3, 107–115, 1935.
- Farber, J. M. Predictive modeling of food deterioration and safety. In *Foodborne Microorganisms and Their Toxins: Developing Methodology*, Pierson, M.D. and Stern, N.J. (Eds.), Marcel Dekker, New York, 1986, pp. 57–90.
- Fernandez, P.S., Baranyi, J., and Peck, M.W. A predictive model of growth from spores of non-proteolytic *Clostridium botulinum* in the presence of different CO₂ concentrations as influenced by chill temperature, pH and NaCl. *Food Microbiol.*, 18, 453–461, 2001.
- Gänzle, M.G., Ehmann, M., and Hammes, W.P. Modeling of growth of *Lactobacillus sanfranciscensis* and *Candida milleri* in response to process parameters of sourdough fermentation. *Appl. Environ. Microbiol.*, 64, 2616–2623, 1998.
- Garcia-Gimeno, R.M., Hervás-Martinez, C., and de Silóniz, M.I. Improving artificial neural networks with a pruning methodology and genetic algorithms for their application in microbial growth predictions in food. *Int. J. Food Microbiol.*, 72, 19–30, 2002.
- Garcia-Gimeno, R.M., Hervás-Martinez, C., Barco-Alcaia, E., Zurera-Cosano, G., and Sanz-Tapia, E. An artificial neural network approach to *Escherichia coli* O157:H7 growth estimation. *J. Food Sci.*, 68, 639–645, 2003.
- Geeraerd, A.H., Herremans, C.H., Cenens, C., and Van Impe J.F. Application of artificial neural networks as a non-linear modular modeling technique to describe bacterial growth in chilled food products. *Int. J. Food Microbiol.*, 44, 49–68, 1998a.
- Geeraerd, A.H., Herremans, C.H., Ludikhuyze, L.R., Hendrickx, M.E., and Van Impe, J.F. Modeling the kinetics of isobaric–isothermal inactivation of *Bacillus subtilis* alpha-amylase with artificial neural networks. *J. Food Eng.*, 36, 263–279, 1998b.
- Geeraerd, A.H., Valdramidis, V.P., Devlieghere, F., Bernaert, H., Debevere, J., and van Impe, J. Development of a novel modelling methodology by incorporating *a priori* microbiological knowledge in a black box approach. *Int. J. Food Microbiol.*, in press.
- Genigeorgis, C.A. Factors affecting the probability of growth of pathogenic microorganisms in foods. *J. Am. Vet. Med. Assoc.*, 179, 1410–1417, 1981.
- Genigeorgis, C., Carniciu, M., Dutulescu, D., and Farver, T.B. Growth and survival of *Listeria monocytogenes* in market cheeses stored at 4°C to 30°C. *J. Food Protect.*, 54, 662–668, 1991.
- George, S.M., Lund, B.M., and Brocklehurst, T.F. The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. *Lett. Appl. Microbiol.*, 6, 153–156, 1988.



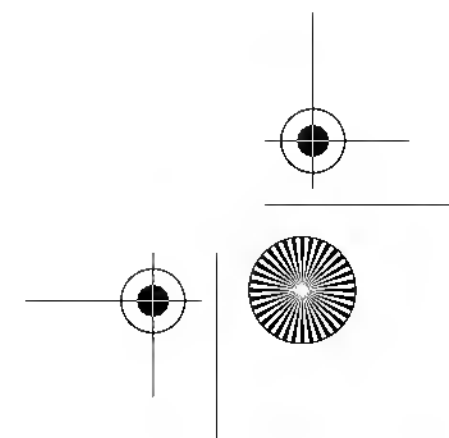
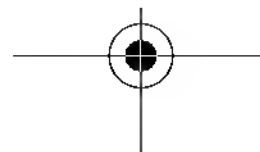
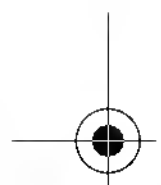
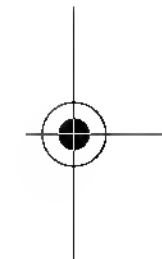
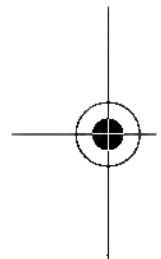


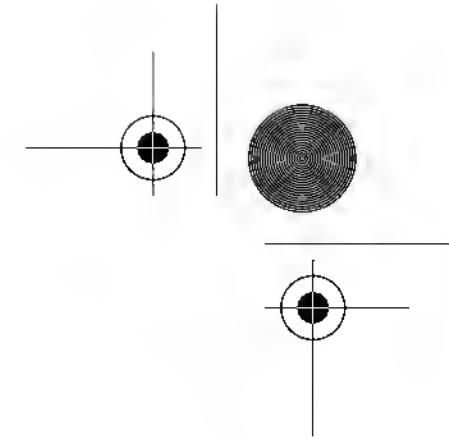
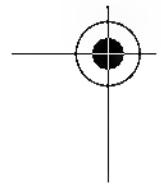
- Gibson, A.M., Bratchell, N., and Roberts, T.A. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *J. Appl. Bacteriol.*, 62, 479–490, 1987.
- Gibson, A.M., Bratchell, N., and Roberts, T.A. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food Microbiol.*, 6, 155–178, 1988.
- Gibson, A.M., Baranyi, J., Pitt, J.I., Eyles, M.J., and Roberts, T.A. Predicting fungal growth: the effect of water activity on *Aspergillus avus* and related species. *Int. J. Food Microbiol.*, 23, 419–431, 1994.
- Gill, C.O. The solubility of carbon dioxide in meat. *Meat Sci.*, 22, 65–71, 1988.
- Giménez, B.C. and Dalgaard, P. Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage microorganisms in cold-smoked salmon. *J. Appl. Microbiol.*, in press.
- Gorris, L. Hurdle technology. In *Encyclopaedia of Food Microbiology*, Robinson, R., Batt, C.A., and Patel, P. (Eds.), Academic Press, London, 2000, pp. 1071–1076.
- Graham, A.F., Mason, D.R., and Peck, M.W. 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, 1996.
- Guillén, M.D. and Errecalde, C. Volatile components of raw and smoked black bream (*Brama rail*) and rainbow trout (*Oncorhynchus mykiss*) studied by means of solid phase microextraction and gas chromatography/mass spectrometry. *J. Sci. Food Agric.*, 82, 945–952, 2002.
- Hajmeer, M.N. and Basheer, I. A probabilistic neural network approach for modeling and classification of bacterial growth/no-growth data. *J. Microbiol. Methods*, 51, 217–226, 2002.
- Hajmeer, M.N. and Basheer, I.A. A hybrid Bayesian-neural network approach for probabilistic modeling of bacterial growth/no-growth interface. *Int. J. Food Microbiol.*, 82, 233–243, 2003a.
- Hajmeer, M.N. and Basheer, I. Comparison of logistic regression and neural network-based classifiers for bacterial growth. *Food Microbiol.*, 20, 43–55, 2003b.
- Hajmeer, M.N., Basheer, I.A., and Najjar, Y.M. Computational neural networks for predictive microbiology. 2. Application to microbial growth. *Int. J. Food Microbiol.*, 34, 51–66, 1997.
- Heitzer, A., Kohler, H.E., Reichert, P., and Hamer, G. Utility of phenomenological models for describing temperature dependence of bacterial growth. *Appl. Environ. Microbiol.*, 57, 2656–2665, 1991.
- Hinshelwood, C.N. Influence of temperature on the growth of bacteria. In *The Chemical Kinetics of the Bacterial Cell*, Clarendon Press, Oxford, 1946, 254–257.
- Hosmer, D.W. and Lemeshow, S. *Applied Logistic Regression*, John Wiley & Sons, New York, 1989.
- Hultin, E. The influence of temperature on the rate of enzymic processes. *Acta Chem. Scand.*, 9, 1700–1710, 1955.
- ICMSF (International Commission on Microbiological Specifications for Foods). *Microorganisms in Foods. 5. Microbiological Specifications of Food Pathogens*, Blackie Academic & Professional, London, 1996a.
- ICMSF. Modelling microbial responses in foods. In *Microorganisms in Foods. 5. Microbiological Specifications of Food Pathogens*, Roberts, T.A., Baird-Parker, A.C., and Tompkin, R.B. (Eds.), Blackie Academic & Professional, London, 1996b, pp. 493–500.



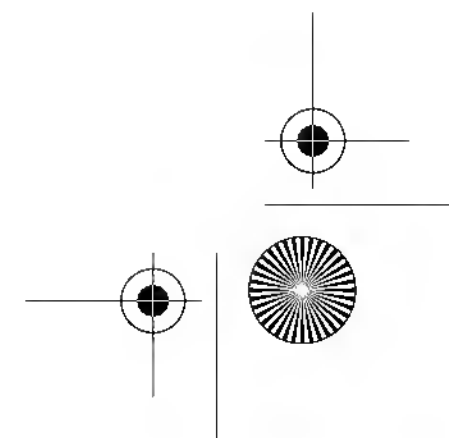
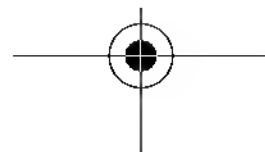
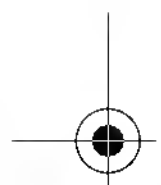
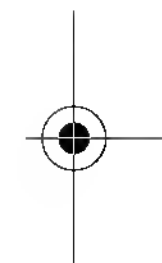
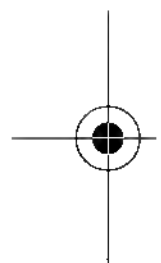


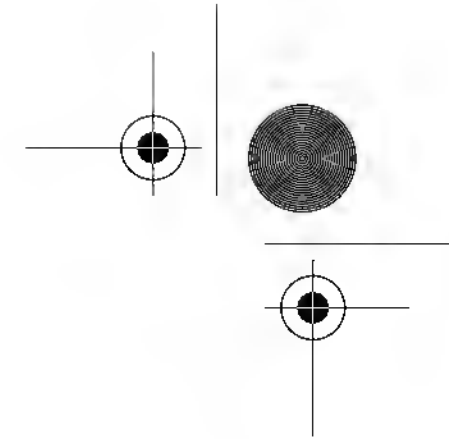
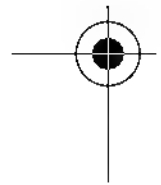
- Ikawa, J.Y. and Genigeorgis, C. Probability of growth and toxin production by nonproteolytic *Clostridium botulinum* in rock sh filets stored under modified atmospheres. *Int. J. Food Microbiol.*, 4, 167–181, 1987.
- James, S. and Evans, J. Performance of domestic refrigerators and retail display cabinets for chilled products. *Refrig. Sci. Technol. Proc.*, 1990–1994, 401–409, 1990.
- Jenkins, P., Poulos, P.G., Cole, M.B., Vandeven, M.H., and Legan, J.D. The boundary for growth of *Zygosaccharomyces bailii* in acidified products described by models for time to growth and probability of growth. *J. Food Prot.*, 63, 222–230, 2000.
- Jeyamkondan, S., Jayas, D.S., and Holley, R.A. Microbial growth modelling with artificial neural networks. *Int. J. Food Microbiol.*, 64, 343–354, 2001.
- Johnson, F.H. and Lewin, I. The growth rate of *E. coli* in relation to temperature, quinine and coenzyme. *J. Cell. Comp. Physiol.*, 28, 47–75, 1946.
- Jones, J.E. and Walker, S. Advances in modeling microbial growth. *J. Ind. Microbiol.*, 12, 200–205, 1993.
- Jones, J.E., Walker, S.J., Sutherland, J.P., Peck, M.W., and Little, C.L. Mathematical modelling of the growth, survival and death of *Yersinia enterocolitica*. *Int. J. Food Microbiol.*, 23, 433–437, 1994.
- Jørgensen, L.V., Dalgaard, P., and Huss, H.H. Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. *J. Agric. Food Chem.*, 48, 2448–2453, 2000.
- Juneja, V.K., Marmer, B.S., Phillips, J.G., and Palumbo, S.A. Interactive effects of temperature, initial pH, sodium chloride, and sodium pyrophosphate on the growth kinetics of *Clostridium perfringens*. *J. Food Prot.*, 59, 963–968, 1996.
- Kalina, V. Dynamics of microbial growth and metabolic activity and their control by aeration. *Antonie Van Leeuwenhoek*, 63, 353–373, 1993.
- Karl, H. Bestimmung des Nitritgehaltes in rucherischen und anderen Fleischprodukten. *Deutsche Lebensmittel-Rundschau*, 2, 41–45, 1992.
- Koutsoumanis, K. Predictive modeling of the shelf life of fish under nonisothermal conditions. *Appl. Environ. Microbiol.*, 67, 1821–1829, 2001.
- Koutsoumanis, K. and Nychas, G.-J.E. Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life prediction. *Int. J. Food Microbiol.*, 60, 171–184, 2000.
- Koutsoumanis, K., Tassou, C.C., Taoukis, P.S., and Nychas, G.-J.E. Modelling the effectiveness of a natural antimicrobial on *Salmonella enteritidis* as a function of concentration, temperature and pH, using conductance measurements. *J. Appl. Microbiol.*, 84, 981–987, 1998.
- Koutsoumanis, K., Lambropoulou, K., and Nychas, G.-J.E. A predictive model for the non-thermal inactivation of *Salmonella enteritidis* on a food model system supplemented with a natural antimicrobial. *Int. J. Food Microbiol.*, 49, 63–74, 1999.
- Koutsoumanis, K.P., Taoukis, P.S., Drosinos, E.H., and Nychas, G.-J.E. Application 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, 2000.
- Lambert, R.J.W., Skandamis, P.N., Coote, P.J., and Nychas, G.-J.E. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.*, 91, 453–462, 2001.
- Lanciotti, R., Sinigaglia, M., Guardini, F., Vannini, L., and Guerzoni, M.E. Growth/no growth interface of *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella enteritidis* in model systems based on water activity, pH, temperature and ethanol concentration. *Food Microbiol.*, 18, 659–668, 2001.



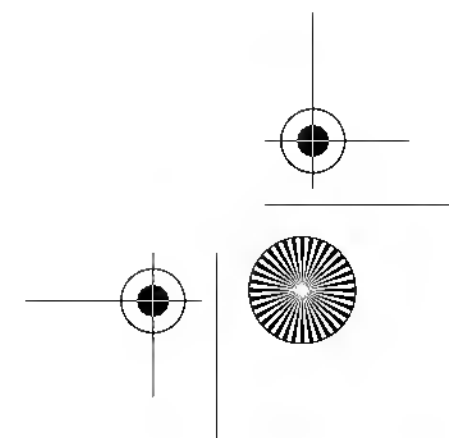
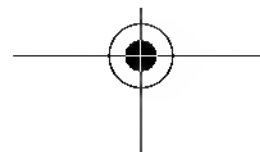
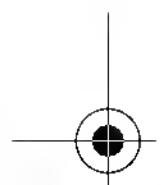
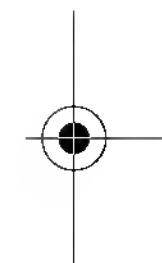
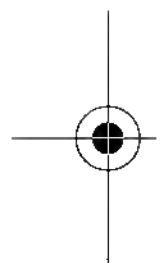


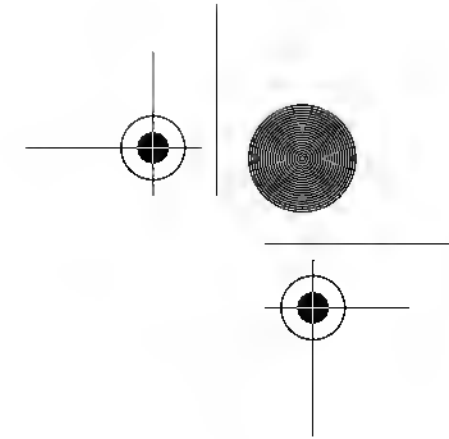
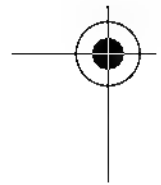
- Legan, D., Vandeven, M., Stewart, C., and Cole, M.B. Modelling the growth, survival and death of bacterial pathogens in food. In *Foodborne Pathogens: Hazards, Risk Analysis, and Control*, Blackburn, C. de W. and McClure, P.J. (Eds.), Woodhead Publishing, Cambridge, England/CRC Press, Boca Raton, FL, 2002, pp. 53–95.
- Leistner, L. Hurdle technology applied to meat products of the shelf stable and intermediate moisture food types. In *Properties of Water in Foods in Relation to Quality and Stability*, Simatos, D. and Multon, J.L., Eds., Martinus Nijhoff Publishers, Dordrecht, 1985.
- Le Marc, Y., Huchet, V., Bourgeois, C.M., Guyonnet, J.P., Mafart, P., and Thuault, D. Modelling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. *Int. J. Food Microbiol.*, 73, 219–237, 2002.
- Le Marc, Y., Pin, C., and Baranyi, J. Methods to determine the growth domain in a multidimensional environmental space. In *Predictive Modeling in Foods—Conference Proceedings*, Van Impe, J.F.M., Geeraerd, A.H., Leguerinel, I., and Mafart, P. (Eds.), Katholieke Universiteit Leuven/BioTec, Belgium, 2003, pp. 34–36.
- Leroi, F. and Joffraud, J.J. Salt and smoke simultaneously affect chemical and sensory quality of cold-smoked salmon during 5°C storage predicted using factorial design. *J. Food Prot.*, 63, 1222–1227, 2000.
- Leroi, F., Joffraud, J.-J., Chevalier, F., and Cardinal, M. Study of the microbial ecology of cold-smoked salmon during storage at 8°C. *Int. J. Food Microbiol.*, 39, 111–121, 1998.
- Leroi, F., Joffraud, J.J., and Chevalier, F. Effect of salt and smoke on the microbiological quality of cold-smoked salmon during storage at 5°C as estimated by the factorial design method. *J. Food Prot.*, 63, 502–508, 2000.
- Leroy, F. and de Vuyst, L. A combined model to predict the functionality of the bacteriocin-producing *Lactobacillus sakei* strain CTC 494. *Appl. Environ. Microbiol.*, 69, 1093–1099, 2003.
- Levenspiel, O. *Chemical Reaction Engineering*, 2nd ed., John Wiley & Sons, New York, 1972.
- Lindroth, S. and Genigeorgis, C. Probability of growth and toxin production by nonproteolytic *Clostridium botulinum* in rock sh stored under modified atmospheres. *Int. J. Food Microbiol.*, 3, 167–181, 1986.
- López-Malo, A., Guerrero, S., and Alzamora, S.M. Probabilistic modelling of *Saccharomyces cerevisiae* inhibition under the effects of water activity, pH and potassium sorbate concentration. *J. Food Prot.*, 63, 91–95, 2000.
- Lou, W. and Nakai, S. Application of artificial neural networks for predicting the thermal inactivation of bacteria: a combined effect of temperature, pH and water activity. *Food Res. Int.*, 34, 573–579, 2001.
- Löwenadler, J. and Rönner, U. Determination of dissolved carbon dioxide by colorimetric titration in modified atmosphere systems. *Let. Appl. Microbiol.*, 18, 285–288, 1994.
- Lund, B.M. Quantification of factors affecting the probability of development of pathogenic bacteria, in particular clostridium botulinum, in foods. *J. Ind. Microbiol.*, 12, 144–155, 1993.
- Lund, B.M., Graham, S.M., and Franklin, J.G. The effect of acid pH on the probability of growth of proteolytic strains of *Clostridium botulinum*. *Int. J. Food Microbiol.*, 4, 215–226, 1987.
- Masana, M.O. and Baranyi, J. Adding new factors to predictive models: the effect on the risk of extrapolation. *Food Microbiol.*, 17, 367–374, 2000a.
- Masana, M.O. and Baranyi, J. Growth/no growth interface of *Brochothrix thermosphacta* as a function of pH and water activity. *Food Microbiol.*, 17, 485–493, 2000b.



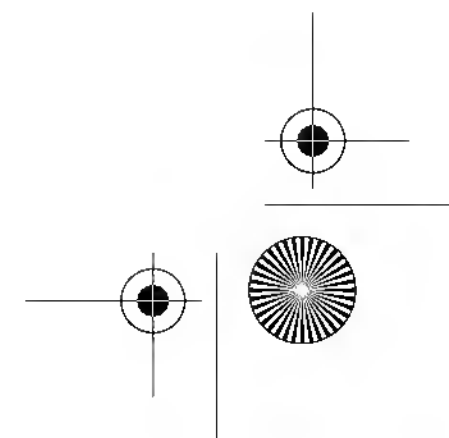
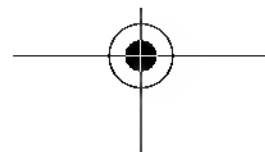
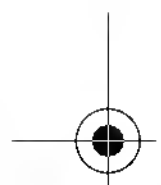
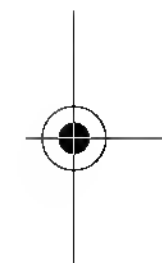
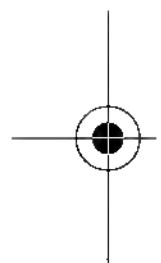


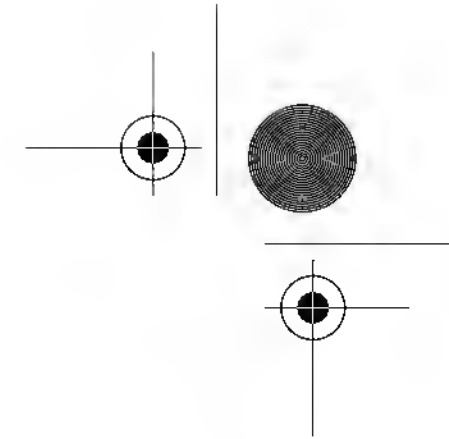
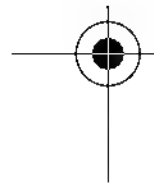
- Mathlouthi, M. Water content, water activity, water structure and the stability of foodstuffs. *Food Control*, 12, 409–417, 2001.
- McClure, P.J., Roberts, T.A., and Otto Oguru, P. Comparison of the effects of sodium chloride, pH and temperature on the growth of *Listeria monocytogenes* on gradient plates and in liquid medium. *Lett. Appl. Microbiol.*, 9, 95–99, 1989.
- McClure, P.J., Baranyi, J., Boogard, E., Kelly, T.M., and Roberts, T.A. A predictive model for the combined effect of pH, sodium chloride and storage temperature on the growth of *Brochotrix thermosphacta*. *Int. J. Food Microbiol.*, 19, 161–178, 1993.
- McClure, P.J., Blackburn, C. de W., Cole, M.B., Curtis, P.S., Jones, J.E., Legan, J.D., Ogden, I.D., and Peck, M.W. Modelling the growth, survival and death of microorganisms in foods: the UK Food Micromodel approach. *Int. J. Food Microbiol.*, 23, 265–275, 1994a.
- McClure, P.J., Cole, M.B., and Davies, K.W. An example of the stages in the development of a predictive mathematical model for microbial growth: the effect of NaCl, pH and temperature on the growth of *Aeromonas hydrophila*. *Int. J. Food Microbiol.*, 23, 359–375, 1994b.
- McDonald, K. and Sun, D.W. Predictive food microbiology for the meat industry: a review. *Int. J. Food Microbiol.*, 52, 1–27, 1999.
- McGill, A.S., Murray, J., and Hardy, R. Some observations on the determination of phenols in smoked fish by solvent extraction: difficulties and improvements in the methodology. *Z. Lebens. Unters. Forsch.*, 181, 363–369, 1985.
- McKellar, R.C. and Lu, X.W. 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, 2001.
- McMeekin, T.A., Chandler, R.E., Doe, P.E., Garland, C.D., Olley, J., Putro, S., and Ratkowsky, D.A. Model for the combined effect of temperature and salt concentration/water activity on growth rate of *Staphylococcus xylosum*. *J. Appl. Bacteriol.*, 62, 543–550, 1987.
- McMeekin, T.A., Olley, J., Ross, T., and Ratkowsky, D.A. *Predictive Microbiology. Theory and Application*, Research Studies Press, Taunton, U.K., 1993.
- McMeekin, T.A., Presser, K., Ratkowsky, D., Ross, T., Salter, M., and Tienungoon, S. Quantifying the hurdle concept by modelling the bacterial growth/no growth interface. *Int. J. Food Microbiol.*, 55, 93–98, 2000.
- Mellefont, L.A. and Ross, T. The effect of abrupt shifts in temperature on the lag phase duration of *Escherichia coli* and *Klebsiella oxytoca*. *Int. J. Food Microbiol.*, 83, 295–305, 2003.
- Mellefont, L.A., McMeekin, T.A., and Ross, T. The effect of abrupt osmotic shifts on the lag phase duration of foodborne bacteria. *Int. J. Food Microbiol.*, 83, 281–293, 2003.
- Mellefont, L.A., McMeekin, T.A., and Ross, T. The effect of abrupt osmotic shifts on the lag phase duration of physiologically distinct populations of *Salmonella typhimurium*. *Int. J. Food Microbiol.*, in press.
- Membré, J.-M. and Burlot, P.M. Effect of temperature, pH, and NaCl on growth and pectinolytic activity of *Pseudomonas marginalis*. *Appl. Environ. Microbiol.*, 60, 2017–2022, 1994.
- Membré, J.M. and Kubaczka M. Degradation of pectic compounds during pasteurised vegetable juice spoilage by *Chryseomonas luteola*: a predictive microbiology approach. *Int. J. Food Microbiol.*, 42, 159–166, 1998.
- Membré, J.M., Thurette, J., and Catteau, M. Modelling the growth, survival and death of *Listeria monocytogenes*. *J. Appl. Bacteriol.*, 82, 345–350, 1997.



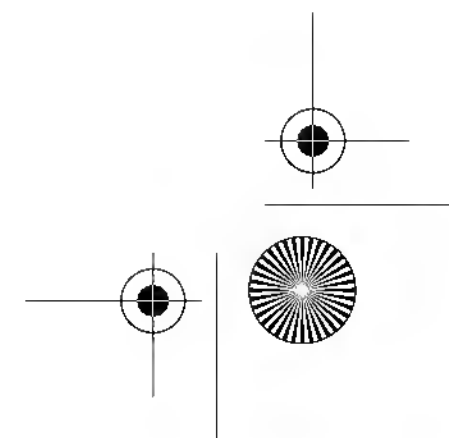
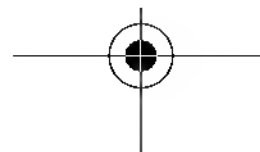
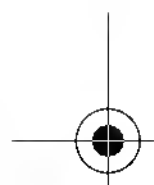
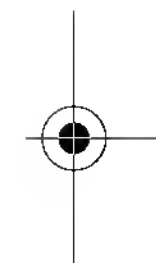
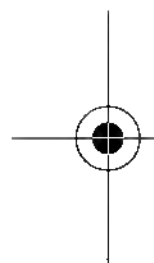


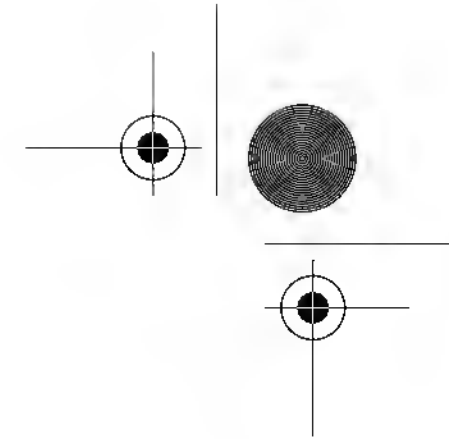
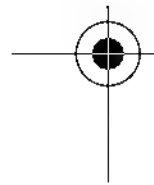
- Membré, J.-M., Ross, T., and McMeekin, T.A. Behaviour of *Listeria monocytogenes* under combined chilling processes. *Lett. Appl. Bacteriol.*, 28, 216–220, 1999.
- Membré, J.M., Kubaczka, M., and Chene, C. Growth rate and growth–no-growth interface of *Penicillium brevicompactum* as functions of pH and preservative acids. *Food Microbiol.*, 18, 531–538, 2001.
- Membré, J.-M., Leporq, B., Vilette, M., Mettler, E., Perrier, L., and Zwietering, M. Experimental protocols and strain variability of cardinal values (pH and a_w) of bacteria using Bioscreen C: microbial and statistical aspects. In *Microbial Adaptation to Changing Environments, Proceedings and Abstracts of the 18th Symposium of the International Committee on Food Microbiology and Hygiene (ICFMH)*, Axelson, L., Tronrud, E.S., and Merok, K.J. (Eds.), MATFORSK, Norwegian Food Research Institute, Ås, Norway, 2002, pp. 143–146.
- Messens, W., Verluyten, J., Leroy, F., and De Vurst, L. Modelling growth and bacteriocin production by *Lactobacillus curvatus* LTH 1174 in response to temperature and pH values used for European sausage fermentation processes. *Int. J. Food Microbiol.*, 81, 41–52, 2002.
- Miles, D.W., Ross, T., Olley, J., and McMeekin, T.A. 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, 1997.
- Miller, M.B. and Bassler, B.L. Quorum sensing in bacteria. *Ann. Rev. Microbiol.*, 55, 165–199, 2001.
- Molina, M. and Giannuzzi, L. Combined effect of temperature and propionic acid concentration on the growth of *Aspergillus parasiticus*. *Food Res. Int.*, 32, 677–682, 1999.
- Najjar, Y.M., Basheer, I.A., and Hajmeer, M.N. Computational neural networks for predictive microbiology. 1. Methodology. *Int. J. Food Microbiol.*, 34, 27–49, 1997.
- Neumeyer, K., Ross, T., and McMeekin, T.A. Development of a predictive model to describe the effect of temperature and water activity on the growth of spoilage pseudomonads. *Int. J. Food Microbiol.*, 38, 45–54, 1997.
- Ng, T.M., Viard, E., Caipo, M.L., Duffy, S., and Schaffner, D.W. Expansion and validation of a predictive model for the growth of *Bacillus stearothermophilus* in military rations. *J. Food Sci.*, 67, 1872–1878, 2002.
- Nichols, D.S., Olley, J., Garda, H., Brenner, R.R., and McMeekin, T.A. Effect of temperature and salinity stress on growth and lipid composition of *Shewanella gelidimarina*. *Appl. Environ. Microbiol.*, 66, 2422–2429, 2002a.
- Nichols, D.S., Presser, K.A., Olley, J., Ross, T., and McMeekin, T.A. Variation of branched-chain fatty acids marks the normal physiological range for growth in *Listeria monocytogenes*. *Appl. Environ. Microbiol.*, 68, 2809–2813, 2002b.
- Nychas, G.-J.E. and Tassou, C.C. Traditional preservatives: oils and spices. In *Encyclopaedia of Food Microbiology*, Robinson, R.K., Batt, C.A., and Patel, P.D. (Eds.), Academic Press, San Diego, 2000, pp. 1717–1722.
- O'Brien, G.D. Domestic refrigerator air temperatures and the public's awareness of refrigerator use. *Int. J. Environ. Health Res.*, 7, 41–148, 1997.
- Oscar, T.P. Response surface models for effects of temperature, pH, and previous growth pH on growth of *Salmonella typhimurium* in brain heart infusion broth. *J. Food Prot.*, 62, 106–111, 1999.
- Oscar, T.P. Development and validation of a tertiary simulation model for predicting the potential growth of *Salmonella typhimurium* on cooked chicken. *Int. J. Food Microbiol.*, 76, 177–190, 2002.
- Palumbo, S.A., Williams, A.C., Buchanan, R.L., and Phillips, J.G. Model for the anaerobic growth of *Aeromonas hydrophila* K144. *J. Food Prot.*, 55, 260–265, 1992.



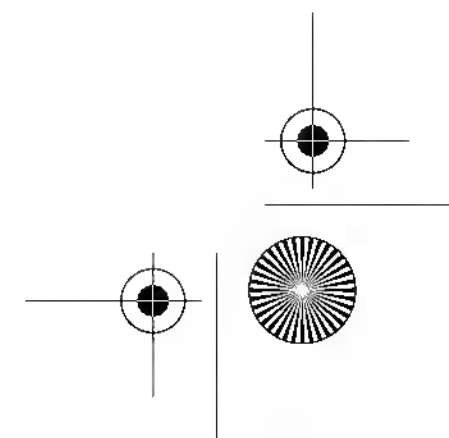
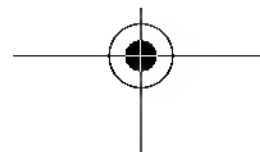
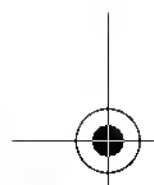
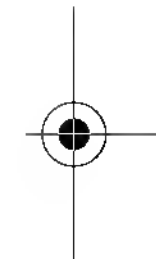
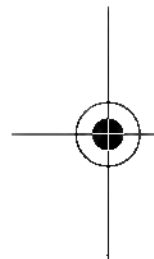


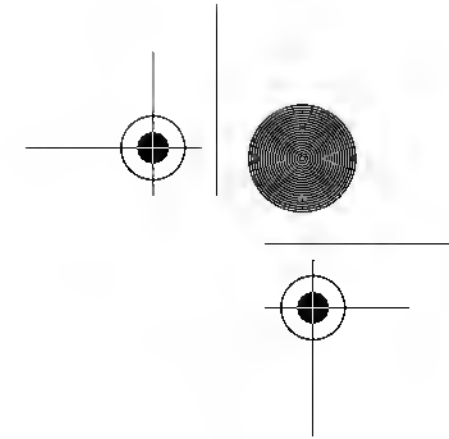
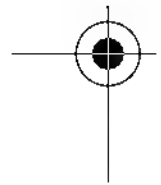
- Palumbo, S.A., Williams, A.C., Buchanan, R.L., Call, J.C., and Phillips, J.G. Expanded model for the aerobic growth of *Aeromonas hydrophila*. *J. Food Saf.*, 16, 1–13, 1996.
- Panagou, E.Z., Skandamis, P.N., and Nychas, G.-J.E. Modelling the combined effect of temperature, pH and a_w on growth of *Monascus ruber*, a heat-resistant fungus isolated from green table olives. *J. Appl. Microbiol.*, 94, 146–156, 2003.
- Parente, E., Giglio, M.A., Ricciardi, A., and Clementi, F. The combined effect of nisin, leucocin F10, pH, NaCl and EDTA on the survival of *Listeria monocytogenes* in broth. *Int. J. Food Microbiol.*, 40, 65–74, 1998.
- Passos, F.V., Fleming, H.P., Felder, R.M., and Ollis, D.F. Modeling growth of *Saccharomyces rosei* in cucumber fermentation. *Food Microbiol.*, 14, 533–542, 1997.
- Passos, F.V., Fleming, H.P., Ollis, D.F., Hassan, H.M., and Felder, R.M. Modeling the specific growth rate of *Lactobacillus plantarum* in cucumber extract. *Appl. Microbiol. Biotechnol.*, 40, 143–150, 1993.
- Pecina, R., Bonn, G., and Burtscher, O. High-performance liquid chromatography elution behaviour of alcohols, aldehydes, ketones, organic acids and carbohydrates on a strong cation-exchange stationary phase. *J. Chromatogr.*, 287, 245–258, 1984.
- Pin, C. and Baranyi, J. Predictive models as means to quantify the interactions of spoilage organisms. *Int. J. Food Microbiol.*, 41, 59–72, 1998.
- Pin, C., Baranyi, J., and de Fernando, G.G. Predictive model for the growth of *Yersinia enterocolitica* under modified atmospheres. *J. Appl. Microbiol.*, 88, 521–530, 2000.
- Pitt, R.E. A descriptive model of mold growth and a toxin formation as affected by environmental conditions. *J. Food Prot.*, 56, 139–146, 1992.
- Pitt, R.E. A model of a toxin formation in stored products. *Trans. ASAE*, 38, 1445–1453, 1995.
- Pouillot, R., Albert, I., Cornu, M., and Denis, J.-B. Estimation of uncertainty and variability in bacterial growth using Bayesian inference. Application to *Listeria monocytogenes*. *Int. J. Food Microbiol.*, 81, 87–104, 2003.
- Presser, K., Ratkowsky, D.A., and Ross, T. Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. *Appl. Environ. Microbiol.*, 63, 2355–2360, 1997.
- Presser, K.A., Ross, T., and Ratkowsky, D.A. Modelling the growth limits (growth/no growth interface) of *E. coli* as a function of pH, lactic acid and temperature. *Appl. Environ. Microbiol.*, 64, 1773–1779, 1998.
- Presser, K.A., Salter, M.A., Ratkowsky, D.A., and Ross, T. (1999). Development of growth limits (growth/no growth interface) modelling and its application to predictive food microbiology. *Recent Res. Dev. Microbiol.*, 3, 535–549, 1999.
- Rasch, M. The influence of temperature, salt and pH on the inhibitory effect of reuterin on *Escherichia coli*. *Int. J. Food Microbiol.*, 72, 225–231, 2002.
- Rasmussen, S.K.J., Ross, T., Olley, J., and McMeekin, T. A process risk model for the shelf-life of Atlantic salmon fillets. *Int. J. Food Microbiol.*, 73, 47–60, 2002.
- Ratkowsky, D.A. *Nonlinear Regression Modeling: A Unified Practical Approach*, Marcel Dekker, New York, 1983, 276 pp.
- Ratkowsky, D.A. Principles of nonlinear-regression modeling. *J. Ind. Microbiol.*, 12, 195–199, 1993.
- Ratkowsky, D.A. Some examples of, and problems with, the use of nonlinear logistic regression in predictive food microbiology. *Int. J. Food Microbiol.*, 73, 119–125, 2002.
- Ratkowsky, D.A. and Ross, T. Modelling the bacterial growth/no growth interface. *Lett. Appl. Microbiol.*, 20, 29–33, 1995.
- Ratkowsky, D.A., Olley, J., McMeekin, T.A., and Ball, A. Relation between temperature and growth rate of bacterial cultures. *J. Bacteriol.*, 149, 1–5, 1982.



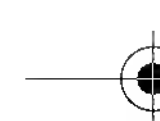
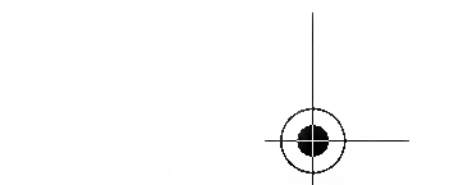
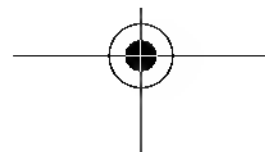
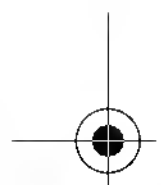
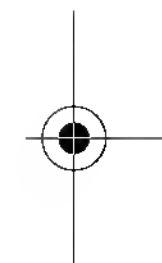
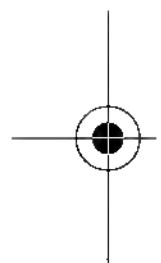


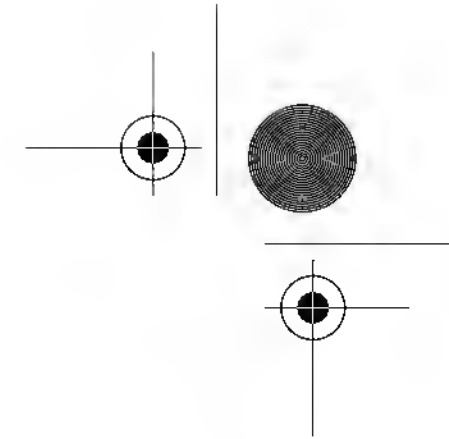
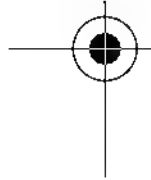
- Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N., and Chandler, R.E. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. *J. Bacteriol.*, 154, 1222–1226, 1983.
- Ratkowsky, D.A., Ross, T., McMeekin, T.A., and Olley, J. Comparison of Arrhenius-type and Bêlehrádek-type models for prediction of bacterial growth in foods. *J. Appl. Bacteriol.*, 71, 452–459, 1991.
- Razavilar, V. and Genigeorgis, C. Prediction of *Listeria* spp. growth as affected by various levels of chemicals, pH, temperatures and storage time in a model broth. *Int. J. Food Microbiol.*, 40, 149–157, 1998.
- Resnik, S.L. and Chirife, J. Proposed theoretical water activity values at various temperatures for selected solutions to be used as reference sources in the range of microbial growth. *J. Food Prot.*, 51, 419–423, 1988.
- Riemann, H. The effect of the number of spores on growth and toxin formation by *C. botulinum* type E in inhibitory environments. In *Botulism, 1966*. Proceedings of the 5th International Symposium on Food Microbiology, Moscow, July 1966, Ingram, M. and Roberts, T.A., Eds., Chapman and Hall, London, 1967, 150–169.
- Roberts, T.A. and Jarvis, B. Predictive modelling of food safety with particular reference to *Clostridium botulinum* in model cured meat systems. In *Food Microbiology: Advances and Prospects*, Roberts, T.A. and Skinner, F.A. (Eds.), Academic Press, New York, 1983, pp. 85–95.
- Robinson, T.P., Ocio, M.J., Kaloti, A., and Mackey, B.M. The effect of growth environment on the lag phase of *Listeria monocytogenes*. *Int. J. Food Microbiol.*, 44, 83–92, 1998.
- Rodriguez, A.M.C., Alcalá, E.B., Gimeno, R.M.G., and Cosano, G.Z. Growth modelling of *Listeria monocytogenes* in packed fresh green asparagus. *Food Microbiol.*, 17, 421–427, 2000.
- Ross, T. *A Philosophy for the Development of Kinetic Models in Predictive Microbiology*, Ph.D. thesis, University of Tasmania, Hobart, Australia, 1993a.
- Ross, T. Bêlehrádek-type models. *J. Ind. Microbiol.*, 12, 180–189, 1993b.
- Ross, T. *Predictive Food Microbiology Models in the Meat Industry*, Meat and Livestock Australia, Sydney, 1999a, 196 pp.
- Ross, T. Assessment of a theoretical model for the effects of temperature on bacterial growth rate. *Refrig. Sci. Technol. Proc.*, EUR 18816, 64–71, 1999b.
- Ross, T. and McMeekin, T.A. Predictive microbiology: a review. *Int. J. Food Microbiol.*, 23, 241–264, 1994.
- Ross, T. and Shadbolt, C.T. *Predicting Escherichia coli Inactivation in Uncooked Comminuted Fermented Meat Products*, Meat and Livestock Australia, Sydney, 2001, 66 pp.
- Ross, T., Fazil, A., and Paoli, G. *Listeria monocytogenes* in Australian processed meat products: risks and their management. *Meat and Livestock Australia*, Sydney, in press.
- Ross, T., Baranyi, J., and McMeekin, T.A. Predictive microbiology and food safety. In *Encyclopaedia of Food Microbiology*, Robinson, R., Batt, C.A., and Patel, P. (Eds.), Academic Press, London, 2000a, pp. 1699–1710.
- Ross, T., Dalgaard, P., and Tienungoon, S. Predictive modelling of the growth and survival of *Listeria* in shery products. *Int. J. Food Microbiol.*, 62, 231–245, 2000b.
- Ross, T., Ratkowsky, D.A., Mellefont, L.A., and McMeekin, T.A. Modelling the effects of temperature, water activity, pH and lactic acid concentration on the growth rate of *Escherichia coli*. *Int. J. Food Microbiol.*, 82, 33–43, 2003.
- Rosso, L. *Modélisation et Microbiologie Prévisionnelle: Élaboration d'un nouvel outil pour l'Agro-alimentaire*, Université Claude Bernard, Lyon, 1995.
- Rosso, L. Models using cardinal values. *Refrig. Sci. Technol. Proc.*, EUR 18816, 48–55, 1999.



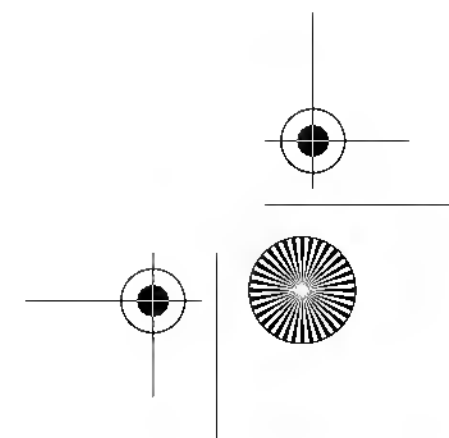
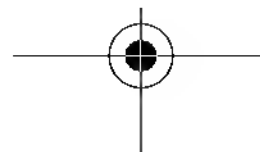
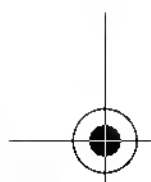
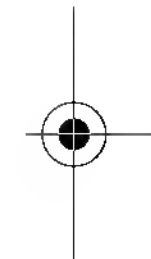
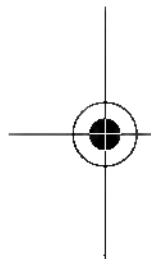


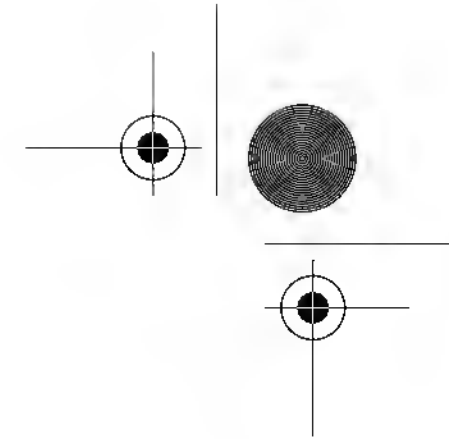
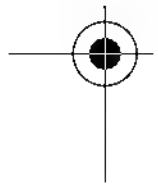
- Rosso, L., Lobry, R., and Flandrois, J.P. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *J. Theor. Biol.*, 162, 447–463, 1993.
- Rosso, L., Lobry, J.R., Bajard, S., and Flandrois, J.P. Convenient model to describe the combined effects of temperature and pH on microbial growth. *Appl. Environ. Microbiol.*, 61, 610–616, 1995.
- Rosso, L. and Robinson, T.P. A cardinal model to describe the effect of water activity on the growth of moulds. *Int. J. Food Microbiol.*, 63, 265–273, 2001.
- Salter, M., Ross, T., and McMeekin, T.A. Applicability of a model for non-pathogenic *Escherichia coli* for predicting the growth of pathogenic *Escherichia coli*. *J. Appl. Microbiol.*, 85, 357–384, 1998.
- Salter, M.A., Ratkowsky, D.A., Ross, T., and McMeekin, T.A. Modelling the combined temperature and salt (NaCl) limits for growth of a pathogenic *Escherichia coli* strain using generalised nonlinear regression. *Int. J. Food Microbiol.*, 61, 159–167, 2000.
- Sautour, M., Dantigny, P., Divies, C., and Bensoussan, M. A temperature-type model for describing the relationship between fungal growth and water activity. *Int. J. Food Microbiol.*, 67, 63–69, 2001.
- Schaffner, D.W. and Labuza, T.P. Predictive microbiology: where are we, and where are we going? *Food Technol.*, 51, 95–99, 1997.
- Schepers, A.W., Thibault, J., and Lacroix, C. Comparison of simple neural networks and nonlinear regression models for descriptive modelling of *Lactobacillus helveticus* growth in pH-controlled batch cultures. *Enzyme Microb. Technol.*, 26, 431–445, 2000.
- Schoolfield, R.M., Sharpe, P.J.H., and Magnuson, C.E. Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. *J. Theor. Biol.*, 88, 719–731, 1981.
- Seman, D.L., Borger, A.C., Meyer, J.D., Hall, P.A., and Milkowski, A.L. Modeling the growth of *Listeria monocytogenes* in cured ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. *J. Food Prot.*, 65, 651–658, 2002.
- Sergelidis, D., Abraham, A., Sarimvei, A., Panoulis, C., Karaioannoglou, P., and Genigeorgis, C. Temperature distribution and prevalence of *Listeria spp.* in domestic, retail, and industrial refrigerators in Greece. *Int. J. Food Microbiol.*, 34, 171–177, 1997.
- Sharpe, P.J.H. and DeMichele, D.W. Reaction kinetics and poikilotherm development. *J. Theor. Biol.*, 64, 649–670, 1977.
- Shewan, J.M. The biological stability of smoked and salted sh. *Chem. Ind.*, July, 501–505, 1949.
- Shoemaker, S.P. and Pierson, M.D. “Phoenix phenomenon” in the growth of *Clostridium perfringens*. *Appl. Environ. Microbiol.*, 32, 803–806, 1976.
- Simpson, R., Almonacid, S., and Acevedo, C. Development of a mathematical model for MAP systems applied to nonrespiring foods. *J. Food Sci.*, 66, 561–567, 2001a.
- Simpson, R., Almonacid, S., and Acevedo, C. Mass transfer in Pacific hake (*Merluccius australis*) packed in refrigerated modified atmosphere. *J. Food Process. Eng.*, 24, 405–421, 2001b.
- Sinigaglia, M., Corbo, M.R., Altieri, C., and Massa, S. Response surface model for effects of temperature, water activity and pH on germination of *Bacillus cereus* spores. *J. Food Prot.*, 22, 121–133, 2002.
- Skandamis, P.N. and Nychas, G.-J.E. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H7 NCTC 12900 in homemade eggplant at various temperatures, pHs, and oregano essential oil concentrations. *Appl. Environ. Microbiol.*, 66, 1646–1653, 2000.



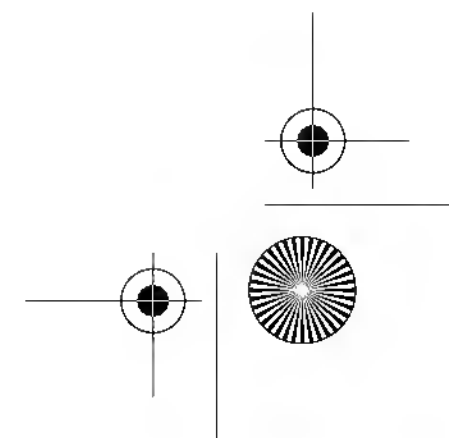
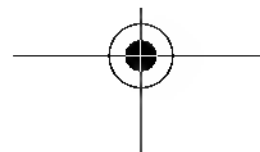
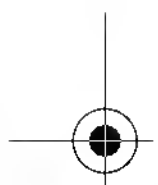
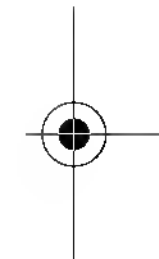
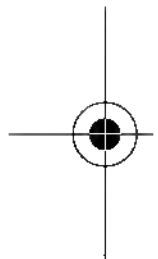


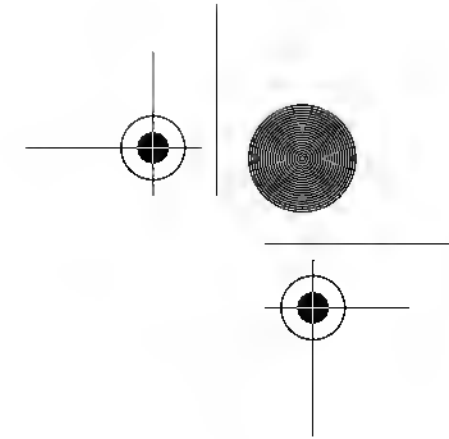
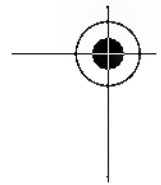
- Skandamis, P.N., Davies, K.W., McClure, P.J., Koutsoumanis, K., and Tassou, C. A vialistic approach for non-thermal inactivation of pathogens in traditional Greek salads. *Food Microbiol.*, 19, 405–421, 2002.
- Skinner, G.E., Lerkin, J.W., and Rhodehamel, E.J. Mathematical modeling of microbial growth: a review. *J. Food Saf.*, 14, 175–217, 1994.
- Smith, M.G. The generation time, lag time and minimum temperature of growth of coliform organisms on meat, and the implications for codes of practice in abattoirs. *J. Hyg. (Camb.)*, 94, 289–300, 1985.
- Sofos, J.N., Beuchat, L.R., Davidson, P.M., and Johnson, E.A. Naturally Occurring Antimicrobials in Food, Task Force Report 132, Council of Agricultural Science and Technology, Ames, IA, 1998.
- Stewart, C.M., Cole, M.B., Legan, J.D., Slade, L., Vandeven, M.H., and Schaffner, D.W. Modeling the growth boundary of *Staphylococcus aureus* for risk assessment purposes. *J. Food Prot.*, 64, 51–57, 2001.
- Stewart, C.M., Cole, M.B., Legan, J.D., Slade, L., Vandeven, M.H., and Schaffner, D.W. *Staphylococcus aureus* growth boundaries: moving towards mechanistic predictive models based on solute specific effects. *Appl. Environ. Microbiol.*, 68, 1864–1871, 2002.
- Suñen, E. Minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic micro-organisms associated with foods. *Lett. Appl. Microbiol.*, 27, 45–48, 1998.
- Sutherland, J.P. and Bayliss, A.J. Predictive modelling of growth of *Yersinia enterocolitica*: the effects of temperature, pH and sodium chloride. *Int. J. Food Microbiol.*, 21, 197–215, 1994.
- Sutherland, J.P., Aherne, A., and Beaumont, A.L. 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, 1996.
- Sutherland, J.P., Bayliss, A.J., and Braxton, D.S. Predictive modelling of growth of *Escherichia coli* O157:H7: the effects of temperature, pH and sodium chloride. *Int. J. Food Microbiol.*, 25, 29–49, 1995.
- Sutherland, J.P., Bayliss, A.J., Braxton, D.S., and Beaumont, A.L. 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, 1997.
- Thurette, J., Membre, J.M., Han Ching, L., Tailliez, R., and Catteau, M. Behavior of *Listeria* spp. in smoked sh products affected by liquid smoke, NaCl concentration, and temperature. *J. Food Prot.*, 61, 1475–1479, 1998.
- Tienungoon, S. *Some Aspects of the Ecology of Listeria monocytogenes in Salmonoid Aquaculture*, Ph.D. thesis. University of Tasmania, Hobart, Australia, 1988.
- Tienungoon, S., Ratkowsky, D.A., McMeekin, T.A., and Ross, T. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl and lactic acid. *Appl. Environ. Microbiol.*, 66, 4979–4987, 2000.
- Tóth, L. and Potthast, K. Chemical aspects of the smoking of meat and meat products. *Adv. Food Res.*, 29, 87–158, 1984.
- Tu, J.V. Advantages and disadvantages of using artificial neural networks versus logistic regression for predicting medical outcomes. *J. Clin. Epidemiol.*, 11, 1225–1231, 1996.
- Tucker, I.W. Estimation of phenols in meat and fat. *Assoc. Off. Agric. Chem.*, 25, 779–782, 1942.
- Tuynenberg Muys, G. Microbial safety in emulsions. *Process Biochem.*, 6, 25–28, 1971.
- Uljas, H.E., Schaffner, D.W., Duffy, S., Zhao, L., and Ingham, S.C. Modeling of combined steps for reducing *Escherichia coli* O157:H7 populations in apple cider. *Appl. Environ. Microbiol.*, 67, 133–141, 2001.





- Valík, L., Baranyi, J., and Görner, F. Predicting fungal growth: the effect of water activity on *Penicillium roqueforti*. *Int. J. Food Microbiol.*, 47, 141–146, 1999.
- Wei, Q.K., Fang, T.J., and Chan, W.C. Development and validation of growth model for *Yersinia enterocolitica* in cooked chicken meats packed under various atmosphere packaging and stored at different temperatures. *J. Food Prot.*, 64, 987–993, 2001.
- Whiting, R.C. Microbial modeling in foods. *Crit. Rev. Food Sci. Nutr.*, 35, 467–494, 1995.
- Whiting, R.C. and Bagi, L.K. Modeling the lag phase of *Listeria monocytogenes*. *Int. J. Food Microbiol.*, 73, 291–295, 2002.
- Whiting, R.C. and Buchanan, R.L. Letter to the editor: a classification of models in predictive microbiology. A reply to K.R. Davey. *Food Microbiol.*, 10, 175–177, 1993.
- Whiting, R.C. and Call, J.E. Time of growth model for proteolytic *Clostridium botulinum*. *Food Microbiol.*, 10, 295–301, 1993.
- Whiting, R.C. and Cygnarowicz-Provost, M. A quantitative model for bacterial growth and decline. *Food Microbiol.*, 9, 269–277, 1992.
- Whiting, R.C. and Golden, M.H. Modeling temperature, pH, NaCl, nitrite and lactate on the survival of *Escherichia coli* O157:H7 in broth. *J. Food Safety*, 23, 61–74, 2003.
- Whiting, R.C. and Oriente, J.C. Time-to-turbidity model for non-proteolytic type B *Clostridium botulinum*. *Int. J. Food Microbiol.*, 36, 49–60, 1997.
- Whiting, R.C. and Strobaugh, T.P. Expansion of the time-to-turbidity model for proteolytic *Clostridium botulinum* to include spore numbers. *Food Microbiol.*, 15, 449–453, 1998.
- WHO/FAO (World Health Organisation and Food and Agriculture Organisation). Risk Assessment of *Listeria monocytogenes* in ready-to-eat foods. Food and Agriculture Organisation, Rome, in press.
- Wijtzes, T., De Wit, J.C., Huis in't Veld, J.H., van't Riet, K., and Zwietering, M.H. Modelling bacterial growth of *Lactobacillus curvatus* as a function of acidity and temperature. *Appl. Environ. Microbiol.*, 61, 2533–2539, 1995.
- Wijtzes, T., van't Riet, K., Huis in't Veld, J.H., and Zwietering, M.H. A decision support system for the prediction of microbial food safety and food quality. *Int. J. Food Microbiol.*, 42, 79–90, 1998.
- Wijtzes, T., Rombouts, F.M., Kant-Muermans, M.L., van't Riet, K., and Zwietering, M. 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, 2001.
- Winans, S.C. and Bassler, B.L. Mob psychology. *J. Bacteriol.*, 184, 873–888, 2000.
- Yan, W. and Hunt, L.A. An equation for modelling the temperature response of plants using only the cardinal temperatures. *Ann. Bot.*, 84, 607–614, 1999.
- Yin, W. and Wallace, D.H. A nonlinear model for crop development as a function of temperature. *Agric. Forest Meteorol.*, 77, 1–16, 1995.
- Zaika, L.L., Moulden, E., Weimer, L., Phillips, J.G., and Buchanan, R.L. Model for the combined effects of temperature, initial pH, sodium chloride and sodium nitrite concentrations on anaerobic growth of *Shigella flexneri*. *Int. J. Food Microbiol.*, 23, 345–358, 1994.
- Zaika, L.L., Phillips, J.G., Fanelli, J.S., and Scullen, O.J. Revisited 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, 1998.
- Zhao, Y., Wells, J.H., and McMillin, K.W. Dynamic changes of headspace gases in CO₂ and N₂ packed fresh beef. *J. Food Sci.*, 60, 571–575, 591, 1995.
- Zhao, L., Chen, Y., and Schaffner, D.W. Comparison of logistic regression and linear regression on modelling percentage data. *Appl. Environ. Microbiol.*, 67, 2129–2135, 2001.
- Zwietering, M. Microbial and engineering characteristic numbers for process evaluation. *Refriger. Sci. Technol. Proc.*, EUR 18816, 272–279, 1999.





- Zwietering, M.H., Cuppers, H.G.A.M., De Wit, J.C., and van't Riet, K. Evaluation of data transformations and validation of models for the effect of temperature on bacterial growth. *Appl. Environ. Microbiol.*, 60, 195–203, 1994.
- Zwietering, M.H., de Koos, J.T., Hasenack, B.E., De Wit, J.C., and van't Riet, K. Modeling of bacterial growth as a function of temperature. *Appl. Environ. Microbiol.*, 57, 1094–1101, 1991.
- Zwietering, M.H., De Wit, J.C., and Notermans, S. Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the time of consumption. *Int. J. Food Microbiol.*, 30, 55–70, 1996.
- Zwietering, M.H., Jongenburger, I., Rombouts, F.M., and van't Riet, K. Modeling of the bacterial growth curve. *Appl. Environ. Microbiol.*, 56, 1875–1881, 1990.
- Zwietering, M.H., Wijtzes, T., De Wit, J.C., and van't Riet, K. A decision support system for prediction of the microbial spoilage in foods. *J. Food Prot.*, 55, 973–979, 1992.

