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Growth kinetics for shelf-life prediction: theory and practice

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SUMMARY

Predictive microbiology can be used to determine and predict the shelf-life of perishable foods under commercial distribution conditions based on microbial growth kinetics. This paper presents general microbial growth kinetics with the Monod model and the Gompertz function. Additional models are given to describe effects of food composition (e.g. a_w) and environmental conditions (e.g. temperature, gas atmosphere) as well as their interaction on the growth kinetic parameters (lag time and specific growth rate). These models can be used to predict the time to reach a critical level under any constant conditions within the range tested. A combination of microbial kinetics with an engineering accumulation approach can be used to predict the final microbial level in a food, or the loss of shelf-life, for any known time-temperature sequence, if there is no history effect or the history effect is negligible. A time-temperature indicator could be used for predicting the remaining shelf-life of perishable foods under any distribution condition based on microbial growth kinetics.

PREDICTIVE MICROBIOLOGY APPROACHES

Microbiological decay is a major mode of food deterioration, especially of fresh products. Spoilage of foods by microbial growth usually requires a high number of cells to be present. The dominant organisms leading to spoilage of a particular product depend on the food composition and the environmental conditions under which the food is stored. For example, in refrigerated packaged beef, *Pseudomonas* spp. were dominant aerobically while *Lactobacillus* and others were dominant anaerobically [107]. Spoiled products, even if no health hazard is present, are considered adulterated under Section 402(a)(1) and 402(a)(3) of the Food, Drug and Cosmetic Act of the United States and thus processors should conduct shelf-life tests to determine when spoilage occurs. On the other hand, foods contaminated with pathogens are not necessarily organoleptically spoiled. If these foods are consumed, they could cause foodborne diseases even when containing a small number of pathogens. Processing, subsequent handling and distribution must ensure absolute control over these pathogens and the manufacturer should verify the effectiveness of the various barriers to prevent their presence and growth in foods using appropriate tests [73].

An emerging type of food processing is the manufacture of extended shelf-life refrigerated (ESLR) foods [53], which may have a shelf-life from ten days to as long as six weeks under proper refrigerated storage conditions. The extension of shelf-life is obtained by strict quality assurance/control (QA/QC) on product formulation and processing based on Hazard Analysis and Critical Control Point (HACCP) principles and by use of various hurdles to microbial growth such as vacuum or modified atmosphere packaging (MAP), antimicrobials, pH reduction, pasteurization and irradiation.

Leistner [61] and Rodel and Leistner [85] introduced the hurdle concept to describe the effects of various intrinsic food composition factors and extrinsic environmental conditions, on microbial growth and survival as listed in Table 1. Quantitative data for the required level of any hurdle

TABLE 1

Food composition factors and environmental conditions affecting microbial growth

Food composition factors	Environmental conditions
pH, acidity % buffering power	Temperature and fluctuation
a_w water content and hysteresis	Relative humidity and fluctuation
Eh (Redox potential)	Light intensity
Antimicrobial agent (natural and additive)	Gases (O_2, CO_2 and their ratio, ethylene)
Microbial flora distribution and competition	Packaging characteristics and interaction
Frozen state	
Physical structure (e.g. emulsion)	
Biological structure (e.g. eggshell)	
Nutrients (lipids, protein, etc.)	
Colloidal constituents	
Surface/volume ratio of substrate	

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and its synergistic effect on other hurdles is limited. Recently, the concept of 'predictive microbiology' has been introduced [3,29,66,84]. Predictive microbiology, through the use of mathematical models, allows for quantification of multiple independent variables and their interactions. From this it becomes quite clear that the graphical representation of the hurdle concept as presented in some papers is not exactly correct. Hurdles should not be viewed as a successive series of obstacles to overcome, rather there is one obstacle, the size of which grows or shrinks as each barrier is introduced or changed in concentration. If hurdles function in a synergistic fashion, then the sum of the effects may be greater than the effect of each added together. However, a number of authors [e.g. 13,23] have stated that their models suggest that the combined effects of temperature and water activity are additive.

The most important hurdle for ESLR foods is proper refrigeration conditions. Variations in temperature throughout the distribution that would be acceptable for 'conventional' refrigerated products are very detrimental to the quality and shelf-life of ESLR foods and can lead to unsafe conditions since pathogens may grow in the absence of normal spoilage organisms. The eventual spoilage of an ESLR product is often caused by quite different types of microorganisms and/or mechanisms compared to those found in the 'conventional' product. There is little information available on microbial based shelf-life of these products as a function of temperature. Accurate determination and prediction of shelf-life of these products are important in terms of safety, legal liability and cost.

Predictive microbiology can be applied to determine and predict the shelf-life of perishable foods under commercial conditions based on microbial growth kinetics. It can also be used to predict the safety of a product which lacks a hurdle against the growth of pathogens and to evaluate the effects of various hurdle factors, including composition, processing and packaging on microbial stability in foods. A key point that must be made is that there are two approaches of predictive microbiology. In the first approach, one does studies of growth under many different conditions, such as was done by Gibson and Roberts [36] for *Clostridium botulinum*. Data at each condition is used to determine a lag time or growth rate parameter and then these data are modeled by some function to give a three-dimensional surface which indicates growth rate or condition for no growth for two environmental factors simultaneously. Multivariant models based on the use of the Gompertz function in combination with response surface analysis have been developed to predict the behavior of foodborne pathogens in response to food formulation and storage parameters, including temperature, pH, sodium chloride content, sodium nitrite concentration, and atmosphere [6,8,9,36]. A similar logistic regression analysis has been used to calculate the probability of toxin production from one spore of *C. botulinum* in fish homogenate as a function of temperature and inoculum size [62]. Regression analysis was also used by Jensen et al. [46] to model growth of *C. botulinum* in laboratory medium and by Ikawa and Genigeorgis [43] for fish fillets stored under modified atmospheres. The probability of growth of *Zygosaccharomyces bailii* in a fruit drink has been modeled similarly [18,19]. These plots are especially useful for determining the needed hurdles to prevent outgrowth of pathogens and spoilage. Large databases are now being established to do this by the USDA, Campden Food and Drink Association and Unilever. The Pathogen Modeling Program developed by the USDA is a database applicable to six pathogens, *Salmonella* spp., *Listeria monocytogenes, Shigella flexneri, Staphylococcus aureus, Aeromonas hydrophila* and *Escherichia coli* 0157:H7. It should be noted that one should not extrapolate beyond the database as all the boundaries may not be established. Unfortunately, these databases are not designed to predict what will happen to the food if environmental conditions such as temperature or relative humidity are varied over time as in a real distribution. This is the second approach of predictive microbiology, i.e. evaluating the growth or loss of shelf-life in a product under a variable storage environment using data from constant environmental conditions.

The main purpose of this paper is to present mathematical models that are available to describe quantitative effects of various hurdles on microbial growth kinetics which can change during food distribution and to demonstrate the applicability of microbial growth kinetics in predicting shelf-life of foods under fluctuating environmental conditions. Aspects of the variability of microbiological data, effects of packaging materials and thermal properties of foods on response of food content to environmental change, time-temperature history effects for microbial growth, will also be discussed wherever appropriate.

MICROBIAL GROWTH KINETICS AND ENVIRONMENTAL HURDLE FACTORS

General growth curve

In general, for any homogeneous microbial population under steady-state conditions, the growth on a nutrient medium or a real food system can be typified by the curve in Fig. 1. The stationary and death phases are generally not applicable to shelf-life as by that time, the food would be unacceptable. Food composition, environmental conditions,

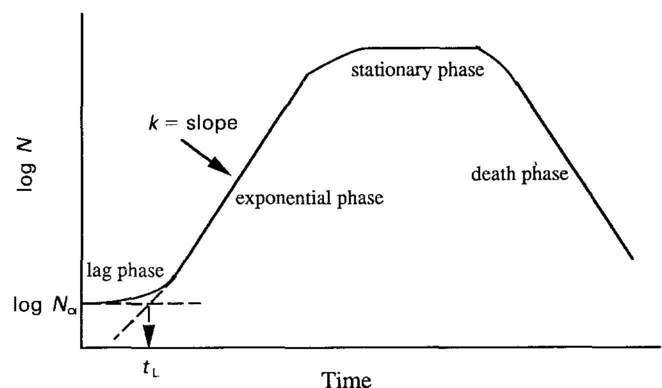

Fig. 1. A typical microbial growth curve.

and age and status (injured or not) of the microbes upon being inoculated or contaminated, may affect the shape of a growth curve as shown in Fig. 2. Growth curve (a) does not include a lag phase, which may occur when the organism is transferred to the same environmental condition at a relatively high population and during the stage of exponential growth. Growth curve (b) is the same as the typical growth curve in Fig. 1. Growth curve (c) has a drop in the population during the lag phase. The degree of the drop depends on the growth stage of the flora upon being transferred and the change of environmental condition(s) and further injury. Curve (d) represents a desirable case, where the net population does not change, i.e. the growth rate is equal to zero, although the microbes may survive in the form of spores or just do not divide, e.g. near the minimum water activity (a_w) for growth. As long as the initial population is low enough, this is not a concern in terms of shelf-life but could be a problem if the organisms were pathogens. Curve (e) is a die-off curve, where microorganisms cannot survive, thus the product is microbiologically stable. There are many descriptive methods proposed in the literature [e.g. 104,110] to describe a microbial growth curve, where the main assumptions are that the population is homogeneous and the growth rate or generation time is age-independent. In this chapter, only the Monod model and the Gompertz function will be discussed in detail.

Since a food usually spoils much before the stationary phase is reached, one should try to maximize analysis of the lag time and the growth rate in the exponential phase. Determination of these two kinetic parameters and modeling of the quantitative effects of various hurdles on them become critical for microbial shelf-life prediction and risk assessment.

Determination of growth kinetic parameters

1. Monod model. Monod [72] stated that the rate at which the population increases is proportional to the number of members in the population, i.e. the specific growth rate or the generation time is assumed to be constant for constant environmental conditions. This is effective within a short

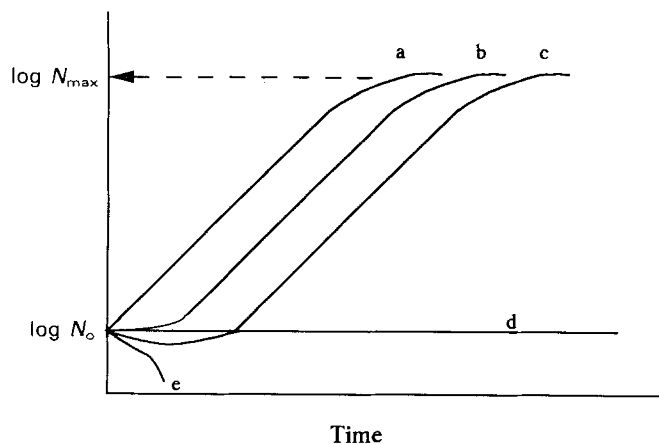

Fig. 2. Various types of microbial growth curves.

period of time, i.e. before the growth begins to significantly decrease the substrate available or change the environment (e.g. lower pH). The integrated form of the Monod model is:

$$N = N_0 \exp[k(t - t_L)] \quad (1)$$

where N is the number of organisms at time t, N_0 is the initial number, k is the specific growth rate, and t_L is the lag time.

The specific growth rate is the slope of the plot of ln N vs t, for $t > t_L$. The lag time, t_L, can be determined graphically, corresponding to the point of intersection of the N_o line with the linear regression line of the exponential growth phase, as shown in Fig. 1. When experimental points show a decline in cell numbers before growth commences, i.e. curve (c) in Fig. 2, the point at which the line describing this decline in cell numbers in the lag phase meets the calculated exponential growth phase is found and this can be used as the lag time value. This model is simple and fairly accurate and has been used extensively, including for mixed flora [107].

2. Gompertz function. A microbial growth curve can also be modeled by a non-linear model, called the Gompertz function, which was first introduced by Gibson et al. [37] to describe microbial growth. Due to space and nutrient limitation, as well as toxic metabolite production, the specific growth rate of microbes is not constant over time, but increases to a maximum, then decreases. The Gompertz function has the form:

$$\text{Log } N = A + C \exp\{-\exp[-B(t-M)]\} \quad (2)$$

where: N is CFU unit^{-1} at time t, A = asymptotic log count of bacteria as time decreases indefinitely, C = asymptotic amount of growth that occurs as t increases indefinitely (i.e. number of log cycles of growth) [log(CFU unit^{-1})], M = time at which the absolute growth rate is maximal, and B = relative growth rate at M.

Lag phase duration, exponential growth rate or generation time as well as maximum population density can be derived from the above four parameters by non-linear regression of log N vs time [8,37]. Zwietering et al. [110] manipulated Eqn 2 so that it contains three biologically relevant parameters, i.e. the maximum specific growth rate, the lag time, and the maximum microbial density. In addition, they made an extensive comparison and confirmed the adequacy of the Gompertz function and its advantage over several other sigmoidal functions. Garthright [34] further made several refinements in the prediction of microbial growth curves and found additional reasons to prefer the Gompertz function. The lag time can also be determined mathematically [7,34].

Both the original and modified Gompertz function have been tested for many species of microorganisms and fits the data very well [8,65]. However, the fit of the Gompertz function is greatly affected by the number of observations

made for the growth curve and the statistical quality of those counts. For a good fit of the function, one needs at least 15 data points spread uniformly through all growth phases. This is a problem if the growth is too fast or the lag time is too short or the experiment ends too early before reaching the stationary phase. The combination of too few observations and variable counts led to failure of the Gompertz function for some data [38]. Reducing the number of points per curve increased the number of curves that could not be fitted [4]. Several protocols have been developed as described elsewhere in the symposium for optimizing the use of the Gompertz function.

3. Comparison of Monod model with Gompertz function. Table 2 gives the comparison of the data from Fu et al. [32] for the growth of *Pseudomonas fragi* in a simulated milk system. As expected, the maximum growth rate constant determined from the Gompertz function is higher than the specific growth rate constant determined from the Monod model. The lag time shows the opposite effect. However, the predicted time to reach 2×10^7 CFU ml^{-1} is almost the same and close to the actual time for both models except at 2 °C, where the difference mainly comes from the lag time determined graphically. This indicates that the models would predict about the same shelf-life for a constant storage temperature. However, the Monod model is easier to use practically than the Gompertz function, despite the fact that software are commercially available for use on a PC or mainframe.

Effect of temperature

Temperature is one of the most important environmental factors affecting the growth and viability of microbes. Although microbial growth can occur at temperatures from about −8 °C to +90 °C, the range of temperature that permits growth of any specific organism seldom exceeds 35 °C [42]. Within this range, temperature affects the duration of the lag phase, the rate of growth, the final cell numbers, the nutrient requirements, and the enzymatic and chemical composition of the food.

Food-poisoning bacteria can multiply within a temperature range from about 0 °C to 50 °C, but only a few (e.g. types of E, F, and non-proteolytic type B strains of *C. botulinum* and *Yersinia enterocolitica*) can grow down to refrigeration temperature [35]. Refrigerated storage of food will favor gram-negative spoilage bacteria or psychrotolerant pathogens, whereas higher-temperature storage may favor growth of *Bacillus cereus, Clostridium perfringens, S. aureus*, and other mesophilic foodborne microorganisms.

1. Arrhenius model. The Arrhenius equation based on thermodynamic considerations has had notable success in describing the temperature dependence of many chemical reactions related to the shelf-life of foods, including loss of sensory quality [51,52]. The growth of a cell is controlled by a chromosome. Division of a cell is usually preceded by a division of the chromosome. But the doubling time could be shorter than the time required to replicate the chromosome through the mechanism of replication forks [41]. Since the replication of the gene is a chemical process, it seems reasonable that the growth would follow the Arrhenius law for a certain temperature range, i.e. the growth could be characterized by an overall activation energy if all other ecological factors were kept constant. The Arrhenius model takes the form:

$$k = k_0 \exp(-E_A/RT) \quad (3)$$

where k is the microbial growth rate constant, k_0 is the 'collision' or 'frequency' factor, T is the absolute temperature (K), R is the universal gas constant (8.314 J mol^{-1} K^{-1}) and E_A (J mol^{-1}) is a thermodynamically defined quantity called the activation energy, which is a measure of the temperature sensitivity of the reaction(s) responsible for microbial growth. This model states that a plot of ln k vs $1/T$ will give a straight line. E_A can be determined from its slope.

Since bacterial growth involves the interaction of a highly complex series of reactions, the resulting Arrhenius plots may deviate markedly from linearity as the temperature

TABLE 2

Comparison of Monod model with Gompertz function for modeling the growth of *Pseudomonas fragi* in a simulated milk[a]

Temperature (°C)	Growth rate (h^{-1})		Lag time (h)		Time (h) to reach 2×10^7 CFU ml^{-1})		
	Monod	Gompertz	Monod	Gompertz	Monod	Gompertz	Actual[b]
2	0.097 ± 0.006	0.115 ± 0.008	40.0	69.8 ± 0.7	152.6	179.6	175.0
4	0.127 ± 0.006	0.153 ± 0.013	23.0	34.2 ± 0.7	100.5	104.6	104.0
7	0.210 ± 0.029	0.262 ± 0.022	16.0	22.8 ± 0.5	58.3	61.8	61.0
10	0.282 ± 0.018	0.333 ± 0.022	11.0	13.9 ± 0.6	45.2	47.8	46.0
13	0.406 ± 0.021	0.476 ± 0.057	8.0	11.1 ± 0.8	29.6	32.9	32.0
16	0.552 ± 0.039	0.667 ± 0.044	5.5	9.3 ± 0.5	22.6	25.9	25.5
22	0.832 ± 0.126	1.022 ± 0.070	4.0	5.9 ± 0.5	15.5	17.0	16.8

[a] Data after '±' are the 95% confidence intervals.
[b] Actual data estimates.

increases or decreases. Some workers [45] showed a linear response of ln k vs $1/T$ in the mid-range of temperature, but at higher and lower temperatures, the specific growth rate was less than the value predicted by the Arrhenius equation as would be expected since the same is found for enzyme activity. Ratkowsky et al. [82] interpreted the microbial response to temperature to be a continuously downward sloping curve throughout the entire suboptimum temperature range and concluded that the Arrhenius relationship was not applicable. Despite this fact, successful applications of the Arrhenius model for predictive microbiology are available in the literature for many different organisms [21,44,69,83,89,101]. Fig. 3 from Fu et al. [32] contains data using the Arrhenius model for *P. fragi*, showing the deviation at high and low temperatures.

The Arrhenius relationship can also be applied to model the temperature-dependence of the lag phase, which would be critical for prediction of the shelf-life under variable temperature conditions where there is an initial low microbial load. The inverse of the lag time (i.e. lag rate) is used to make the Arrhenius plot [2,32,93,101]. The fit for lag time is usually not as good as for the growth rate constant data from the exponential phase. This is shown in Fig. 4 from the data of Fu et al. [32].

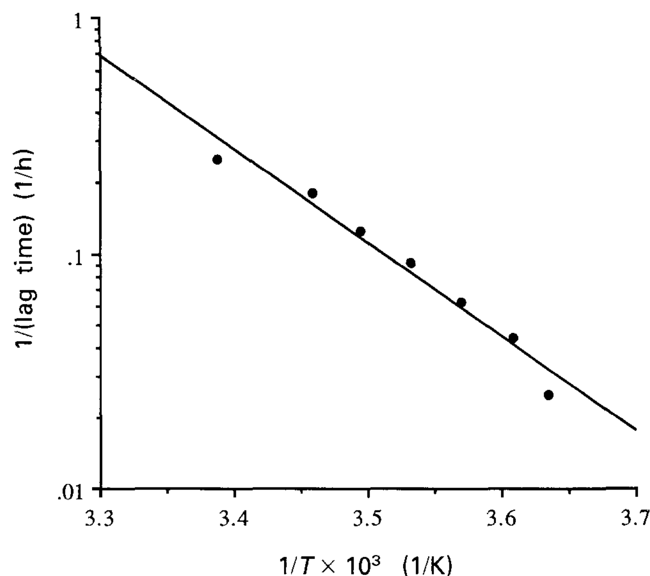

Fig. 4. Arrhenius plot of inverse lag time (lag rate) vs $1/T$ for *Pseudomonas fragi* growth from the results of Fu et al. [32].

2. Square root model. Ratkowsky et al. [82] proposed a simple two-parameter empirical equation for the temperature-dependence of microbial growth up to the optimum temperature (T_{opt}) as:

$$\sqrt{k} = b\ (T - T_{min}) \quad (4)$$

where k is the specific growth rate from the growth curve as before, b is the slope of the regression line of $\sqrt{k}$ vs temperature, T is the test temperature (in either °C or K) and T_{min} is the notational microbial growth temperature where the regression line cuts the temperature axis at $\sqrt{k} = 0$. Ratkowsky et al. [82] applied Eqn 4 to more than 50 sets of growth data with excellent fit, and the equation also accurately described the growth rate data of an additional 30 organisms studied by Ratkowsky et al. [81]. Fig. 5 shows the same data of Fu et al. [32] as in Fig. 3, plotted by the square root function, showing a better fit than found by the Arrhenius relationship.

Ratkowsky et al. [81] expanded the above basic equation to cover the whole biokinetic temperature range and to account for the drop in growth rate above the T_{opt}. The empirical non-linear regression model is:

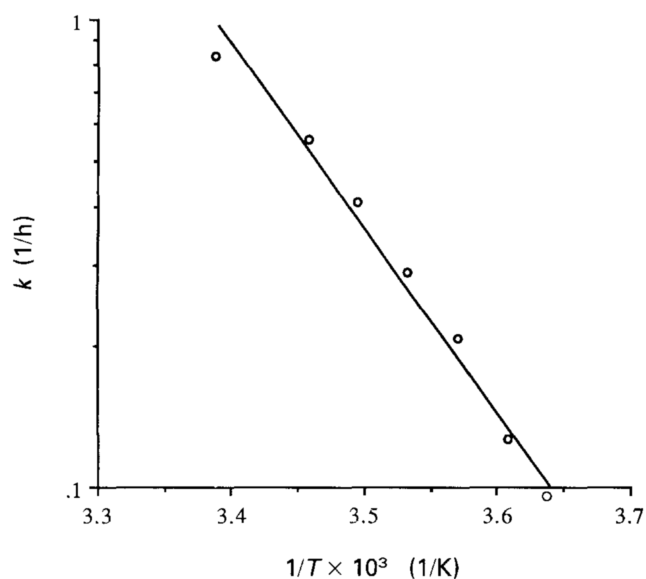

Fig. 3. Arrhenius plot (log k vs $1/T$) for *Pseudomonas fragi* growth from the results of Fu et al. [32].

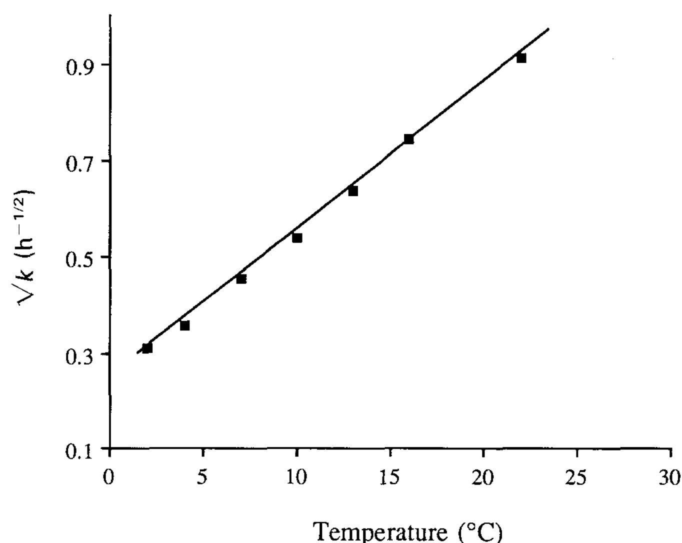

Fig. 5. Square root plot of growth rate constant vs temperature for *Pseudomonas fragi* from the results of Fu et al. [32].

$$\sqrt{k} = b\ (T - T_{min})\ \{1 - \exp\ [c\ (T - T_{max})]\} \quad (5)$$

where b is the regression coefficient of $\sqrt{k}$ vs T (in K) for $T < T_{opt}$, c is an additional parameter to enable the model to fit the data for temperatures above the T_{opt}, and T_{max} is the upper temperature where regression line cuts the temperature axis at $\sqrt{k} = 0$. Zwietering et al. [109] modified Eqn 5 to make it applicable for temperatures above T_{max} and the modified equation was selected as the most suitable model for the specific growth rate as a function of temperature in comparison to several other models.

Since obtaining accurate data at very low growth rates is difficult, T_{min} and T_{max} are notational temperatures and may not be the actual zero growth temperatures. In addition, the calculated T_{min} may be below the freezing point of the food. Freezing would alter the water activity and also affect the growth rate causing deviation from linearity. Since the actual T_{min} for growth may occur at several degrees above the extrapolated value [45], the shelf-life predicted near the lower extreme of growth temperature based on the Square root equation, would be less than actually would occur.

Chandler and McMeekin [12] used the Square root model to describe the effect of temperature on the lag phase of growth and showed that a T_{min} value was obtained similar to that obtained for the log phase of growth (−5.8 °C and −5.2 °C, respectively). Smith [94] has also determined the effect of temperature on the lag and generation times of *E. coli*. Analysis of the data indicate that both parameters obeyed the Square root model and yielded similar T_{min} values (1.75 °C and 2.6 °C).

3. Comparison of Arrhenius model and Square root model. McMeekin et al. [68] pointed out that the nonapplicability of the Arrhenius law to the modeling of the temperature-dependence of microbiological growth can be viewed as resulting from the value of E_A changing with temperature. Activation energy was related to the Square root model by the expression:

$$E_A = 2\ RT^2/(T - T_{min}) \quad (6)$$

Thus for a given organism, the change of E_A is greater for low values of $T - T_{min}$ (ranging from 5 to 30 °C) than for higher values. However, the true temperature-dependence of the activation energy may be incorrect, since the pre-exponential factor (k_0) in the Arrhenius equation may also be temperature-dependent. Overall, for a narrow temperature range, the activation energy can be assumed constant.

Table 3 lists the results for the Arrhenius and Square root model for both the lag time and the specific growth rate constant data of *P. fragi* in a simulated milk system from [32] used in the previous plots. For both cases there was a good fit as would be expected if enough data points are collected.

4. Linear model. Spencer and Baines [95] proposed a linear model based on the research of microbial growth on white fish. They postulated that the effect of temperature on the rate of spoilage of fish stored at a constant temperature between −1 and 25 °C was found to be approximately linear and could be expressed in the form:

$$k = k_0\ (1 + cT) \quad (7)$$

where k = specific spoilage rate (spoilage units day^{-1}) at storage temperature T; k_0 is the specific spoilage rate at 0 °C and c = temperature constant (linear temperature response). Thus a plot of k vs T gives a straight line. Such a response would be expected if both the temperature range and the E_A were small, thus this equation has limited use.

5. Log shelf-life model. If the temperature range of concern is about 20–30 degrees, then a simple plot of the shelf-life (e.g. time to some value of N) on semilog graph paper vs temperature is also a straight line. The log shelf-life equation takes the form of:

$$t_s = t_0\ \exp(-bT) \quad (8)$$

where t_s is the shelf-life at temperature T in °C, t_0 is the shelf-life at 0 °C, b is the slope of a plot of ln t_s vs T. This plot can be used to model the various effects of process, composition and package conditions as well as to establish the temperature sensitivity of the product. Fig. 6 shows a semilog plot of the end of shelf-life of pasteurized milk at different storage temperatures. The time for the psychrotrophic microbial count to reach 10^6 CFU ml^{-1} is superimposed [55]. As seen there is a good correlation of growth and shelf-life.

From this plot a simple approach for temperature-dependence is to use the value of Q_{10}, which is defined as the decrease in shelf-life for a 10 °C increase in temperature. It has been used to predict quality or nutrient losses for many foods and potency degradation for drugs [20,51]. It would be useful to know the theoretical as well as the practical limits of this extrapolating factor in view of both regulatory and economic issues. A difference of 0.5 in the Q_{10} value can have a large effect on the predicted shelf-life. The Q_{10} is usually assumed constant over a narrow range of temperature. From Fig. 6, the calculated Q_{10} is about 4.4. Reported Q_{10}s for microbial growth under refrigeration conditions range from 2 to 10.

There are many other temperature-dependent models for microbial growth rate in the literature [56,109]. However, in general, they have more parameters to be estimated, which makes it very difficult to use for prediction under variable temperature conditions. Zwietering et al. [109] also proposed a hyperbolic model for the description of the lag time as a function of temperature. Dickson et al. [25] used exponential-decay models to describe the effect of temperature on generation time and lag time parameters derived from the Gompertz function.

TABLE 3

Kinetic parameters determined for *Pseudomonas fragi* in a simulated milk[a]

Arrhenius model:		
exponential phase	$\ln k = 30.10 - 8.90 \times 10^3 (1/T)$,	$r^2 = 0.984$
	$\ln k_0 = 30.10 \pm 3.65$	$E_A = 73.90 \pm 8.61$ kJ mol^{-1}
lag phase	$\ln (1/t_L) = 29.90 - 9.17 \times 10^3 (1/T)$,	$r^2 = 0.963$
	$\ln k_0 = 29.90 \pm 7.34$,	$E_A = 76.20 \pm 17.26$ kJ mol^{-1}
Square root model:		
exponential phase	$\sqrt{k} = 0.0306 (T + 7.85)$	$r^2 = 0.998$
	$b = 0.0306 \pm 0.0016$,	$T_{min} = -7.85 \pm 0.24$ °C
lag phase	$1/\sqrt{t_L} = 0.0172 (T + 7.65)$,	$r^2 = 0.993$
	$b = 0.0172 \pm 0.0017$,	$T_{min} = -7.65 \pm 0.45$ °C

[a] From Fu et al. [32].

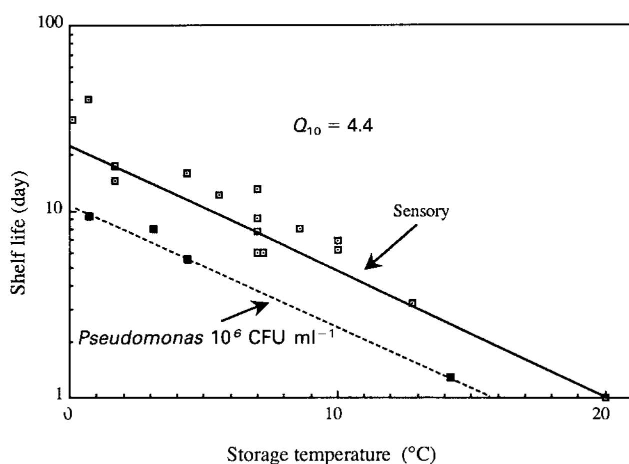

Fig. 6. Shelf life plot for pasteurized milk [55].

Effect of water activity

The growth and metabolism of microbes demands the presence of water in an available form. Fresh foods such as meat, fish, poultry, fruits and vegetables have a_ws above 0.98 and will support the growth of most microorganisms. The a_w in a food may be reduced by increasing the concentration of solutes in the aqueous phase of food, either by removing water or adding solutes, such as salt and sugar in a sauce covering the food or in the food itself.

Many microorganisms, including the pathogenic bacteria, grow most rapidly at a_w levels in the range of 0.980–0.995. For all microbes there is a minimum water activity below which they cannot grow. Bacteria generally require a higher a_w for growth than yeasts, while molds can tolerate much lower a_ws. Gram-positive bacteria tolerate lower a_ws than gram-negative [42]. The method of adding water to a food system may affect the growth response of microorganisms since, usually, a food prepared by a desorption technique has a higher moisture content at a given a_w than the one prepared by an adsorption method [1].

1. Log shelf-life model. As the a_w of the surface or the relative humidity in contact with the surface of a food is decreased, the microbial growth rate decreases thus changing shelf-life. A semilog plot of the shelf-life of cake products based on onset of molding has been found to be a linear function of a_w at constant temperature and oxygen [88]. The log shelf-life equation can be written as:

$$\log \Theta = a + b\, a_w \tag{9}$$

where Θ is the time for mold outgrowth, a and b are constants, corresponding to the intercept and the slope from the plot of log Θ vs a_w. There is at present no theoretical model for this. The data of Kang et al. [49] for the effect of a_w on generation time of *C. perfringens* at high a_w in different humectant-containing media (sucrose vs glycerol), also shows an appropriate linear relationship as seen in Fig. 7.

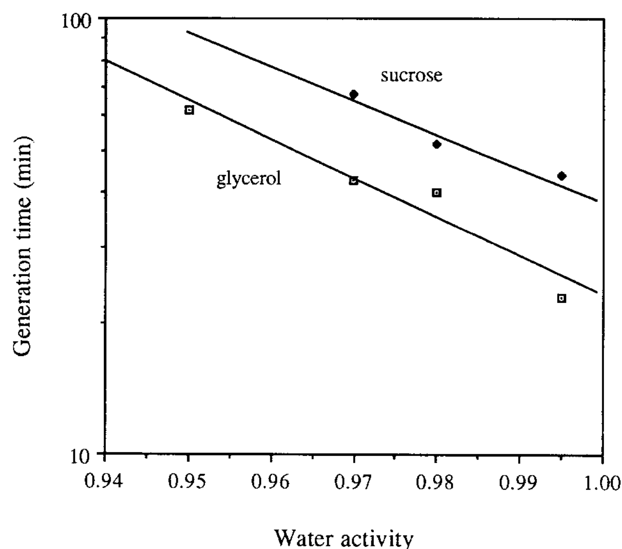

Fig. 7. Effect of water activity on the generation time of *Clostridium perfringens* [49].

2. Temperature/a_w interaction models. Davey [24] modified the Arrhenius equation to produce a model which predicts the combined effect of temperature and water activity on the growth rate of bacteria and which has the form:

$$\ln k = C_0 + C_1/T + C_2/T_2 + C_3a_w + C_4a_w^2 \quad (10)$$

where C_0 through C_4 are the five coefficients to be determined by multiple-linear regression curve fitting techniques. The added constants can be looked at as 'virial coefficients', a term used by physical chemists to describe the higher order effects of factors (a_w, T) on reactions. In essence these virial coefficients are curve fitting terms. Of course, with five constants as in Eqn 10, most models will give good fit. When $a_w \approx 1$, Eqn 10 can be simplified to account for only the effect of temperature. Most refrigerated tissue foods would require a significant decrease in moisture content to drop the a_w significantly and to have an impact in this equation. This equation has also been used for lag time data, as long as $1/t_L$ is substituted for k [24].

McMeekin et al. [67] and Chandler and McMeekin [13] modified the basic Square root equation to incorporate a_w:

$$\sqrt{k} = c\sqrt{(a_w - MINa_w)}\ (T - T_{min}) \quad (11)$$

where c is a constant and $MINa_w$ is the theoretical minimum a_w for the growth of *Staphylococcus xylosus*. This equation has also been used successfully to model the T/a_w responses of other bacteria [87].

In another study, Chandler and McMeekin [14] found that the growth of *Halobacteria* on products such as salted, dried fish, at all water activities below 0.85, could be based on the square root temperature response without the need to incorporate a water activity term. Broughall et al. [5] developed other mathematical models that describe the effects of lowering the water activity on the growth kinetics of *S. aureus* and *Salmonella typhimurium*. Unfortunately, no tests exist for the predictive ability of any of these models under conditions where both temperature and water activity continuously change with time, as would occur with moisture loss or gain during storage, and which is the real test of any predictive equation.

Effect of gas composition

Use of modified atmospheres through direct flushing or by the use of scavengers/emitters (CAP/MAP technology) is now an important area being examined and exploited for food processing and packaging of refrigerated foods [53]. Thus there is a need to examine what effects a change in gas composition will cause on microbial growth rates.

1. Oxygen. The effect of oxygen on the redox potential generated will control microbial growth depending upon the specific microorganism of concern [47]. Restricting oxygen will generally restrict the growth of gram-negative, aerobic spoilage organisms such as *Pseudomonas*, while enhancing the growth of gram-positive, microaerophilic species such as *Lactobacillus* or *Brothothrix*. Facultative anaerobes (gram-positive or negative) are generally unaffected by lack of oxygen and could become predominant (e.g. *Aeromonas*). Table 4 provides some details about the oxygen requirements of microorganisms. Clark and Burki [16] showed the effect of O_2 concentration on the cell growth rate of spoilage organisms (*Pseudomonas* and *Achromobacter*). Above 1.0% O_2, there was no effect on the rate of growth up to 21% O_2, but below 1%, the growth rate slowly fell with about a 20% decrease for a 0.5% decrease in O_2 level. Data such as this for both spoilage and pathogen growth rates in the same food at different temperatures are needed to determine the effect on the food and its eventual safety. Further, microenvironments (especially in a food with a heterogeneous structure) may exist where oxygen tensions are low enough to permit the growth of pathogenic anaerobes, even though the oxygen level of the bulk phase might suggest otherwise [47]. The change in gas phase composition with time makes modeling and prediction very difficult.

Since tissue foods utilize oxygen to some degree (by respiration, oxidation or microbial action) and since most films are permeable to gases like oxygen, one could expect a change in headspace oxygen concentration over time. The same models for gas permeation of moisture through films as elaborated on by Labuza and Contreras-Medelin [54] could be used to establish the oxygen content with time as has been recently done by Mannapperuma et al. [64]. Fig. 8 shows a general relationship between the food oxygen consumption rate and the permeability of the package to oxygen as a function of the internal oxygen level in the package. In essence, regardless of the initial oxygen level, the package interior will always end up at some equilibrium oxygen level (PO_2*) in a matter of a few hours or days [48]. If the oxygen level is below the critical oxygen concentration,

TABLE 4

Oxygen requirements of microorganisms[a]

Aerobes—	require oxygen for growth: Pseudomonads *Acinetobacter/Moraxella* *Micrococcus* Film yeasts Molds
Microaerophiles—	require low levels of oxygen: *Campylobacter* *Lactobacillus*
Facultative organisms—	grow in the presence or absence of oxygen: *Brochothrix thermosphacta* *Staphylococcus* *Bacillus* sp. Enterobacteriaecea *Vibrio* Fermentative yeasts
Anaerobes—	inhibited by oxygen: *Clostridium Botulinum* *Clostridium perfringens*

[a] From Lambert et al. [57].

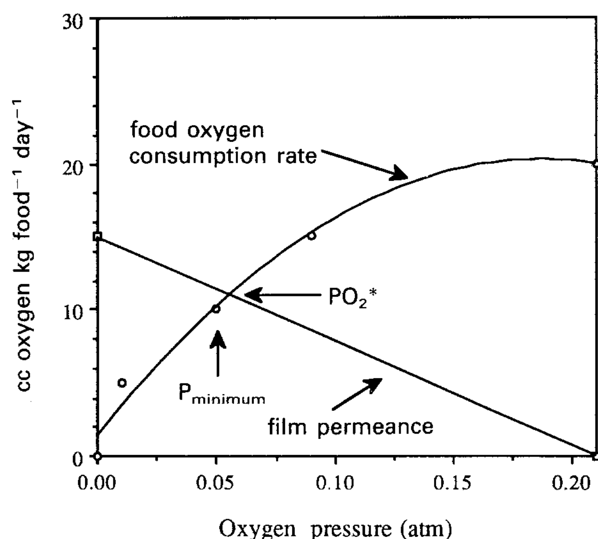

Fig. 8. Generalized oxygen consumption and permeation rates as a function of internal package oxygen pressure. PO_2* represents the critical oxygen level the package will attain.

fresh foods will go into anaerobic glycolysis and rapid biochemical activity will cause rapid spoilage. Thus pathogens would not be a problem because metabolic off-odors would develop first. If the oxygen level is above the critical value, some facultative anaerobic pathogens might grow if the oxygen pressure is not high enough to prevent their growth. At higher oxygen levels, aerobic pathogens may grow, if the competitors are eliminated by some other microbial hurdles. There has been little application of these models or the testing of them under variable temperature and humidity conditions. Since the film permeation rates and the respiration rates are temperature- and relative humidity-dependent, predictions may become impossible for a variable environment. Factors such as oxygen solubility in the food formulation, processing conditions and processing temperature are important. In addition to film oxygen permeability, data on head space volume, package surface area, and oxygen consumption rates due to combined effects of microbial load, respiration rate and chemical oxidation rate are needed.

Transfers between the product-package system and its environment also can have significant consequences on the microbial activities necessarily associated with packaged foods. When beef, pork or lamb meat is wrapped in a permeable film (PVC, permeability to O_2 equal to 10 000 ml m^{-2} day atm), *Pseudomonas* became predominant at 4 °C (42–60%). However, using a more gas-tight film with a permeability to O_2 equal to 5–90 ml m^{-2} day atm, and a permeability to CO_2 of 20–300 ml m^{-2} day atm, *Pseudomonas* was inhibited and lactic acid bacteria became predominant [10].

2. Carbon dioxide. Carbon dioxide (CO_2) may kill, stimulate, inhibit or have no noticeable effect on the growth rate of microorganisms, depending on the organism, the concentration of CO_2, the temperature of incubation, the age of the cells when CO_2 is applied, and the a_w of the food or medium. In general, carbon dioxide extends the lag phase and reduces the growth rate of many microorganisms. The earlier the application of CO_2, the higher its concentration in the atmosphere (0–25%), the lower the load of microorganisms, and the lower the temperature, the greater the inhibitory action by CO_2 [35].

The effect of CO_2 on increasing lag time has been shown for *Pseudomonas aeruginosa* growing on a variety of substrates [50]; *Pseudomonas* and *Achromobacter* species between 0 and 22 °C [16,17]; *Bacillus subtilis* at 30 °C [78]; *Pseudomonas* growing on meat [92]; *S. typhimurium*, enterococci, and *S. aureus* at 10 °C but not at 20 °C [91]; and aerobic *Pseudomonas* species in fish at 4 °C [103]. In contrast, there was no effect of carbon dioxide on the lag time of *Pseudomonas putida* at 25 °C [71], *P. fragi* [28] or *S. aureus* at 30 °C [78].

Thus an effect on the lag time is a common but by no means a universal observation, which makes modeling of growth very difficult. Some evidence suggests that this effect is temperature-dependent, with greater inhibition at lower temperatures [91]. Wodzinski and Frazier [106] found that, at low (5–10%) carbon dioxide levels, the microbial lag time could be increased or decreased relative to air-grown cultures, depending on salt concentration and temperature. Presence of carbon dioxide caused an increase of lag time, which was more evident at limiting a_w and optimum growth temperature.

Carbon dioxide can also reduce the growth rate in the exponential phase of some bacteria [26,27,70]. An enhancement of the effect at lower temperatures has been noted, but at least in the case of *P. fragi* this could be accounted for by the increased solubility of the gas. An exception of this appears to be *B. cereus*, where the maximum inhibition of the growth rate occurred at the optimum rather than the minimum growth temperature [27]. It should be noted, however, that the *Bacillus* were grown anaerobically while the *Pseudomonads* were grown aerobically.

Molin [70] observed that growth rates were inhibited anaerobically at 25 °C by 100% carbon dioxide, but considerable variation occurred among species of ten facultative anaerobes. *Lactobacillus* species were the most resistant to carbon dioxide, but several *Enterobacteriaceae*, although inhibited by carbon dioxide, were still able to grow anaerobically in the presence of carbon dioxide at a greater rate than lactic acid bacteria. In this study, *B. cereus* was found to be the most sensitive to carbon dioxide. A different order of sensitivity was reported in another study [26], which complicates any modeling based on literature data especially because of the lack of data on pathogens.

Lambert et al. [58] studied the effect of headspace CO_2 concentration and irradiation doses on toxin production by *C. botulinum* (Type A, B) in inoculated fresh pork. Toxin production occurred faster in samples initially packaged with 15–30% of CO_2 while higher levels of CO_2 (45–75%) delayed toxin production. Low-dose irradiation delayed toxin production at all levels of CO_2 in the package headspace.

A CO_2 absorbent in the package enhanced toxin production by *C. botulinum*. This was attributed to production of H_2 by the CO_2 absorbent, possibly resulting in a decrease in the oxido-reduction potential of the meat.

As with oxygen, any modeling of the effect of carbon dioxide on shelf-life or food safety due to pathogen growth will be very dependent on the food composition, gas composition level, temperature and changes in water activity. Zhao et al. [108] found that the activation energy, for the growth of *L. monocytogenes* and *Pseudomonas fluorescens* on chicken meat under modified atmosphere storage, was related to the ratio of $[O_2]/(1 + [CO_2])$ for O_2 concentration from 0 to 21% and CO_2 from 0.03 to 80%.

PREDICTION UNDER FLUCTUATING ENVIRONMENTAL CONDITIONS

Temperature abuse/variation is the most important factor in foodborne illness outbreaks, but to what extent the level of temperature abuse or fluctuation increases the risk of foodborne illness as well as the extent to which actual temperature fluctuations decrease shelf-life is not known. The relevant database in the literature for fluctuating temperature conditions is minimal compared to kinetic data and predictive equations available for destruction of microorganisms under variable heat processing conditions.

A major question for CAP/MAP foods is whether organoleptic spoilage due to chemical or microbial action will occur before the number of pathogens or the toxin level becomes a risk when a product undergoes temperature abuse. Since different metabolic pathways and different organisms are affected differently by temperature fluctuation, this creates a real problem in terms of the ability to predict. Generally, the Arrhenius model or the Square root model should be accurate enough to predict the rate of growth and the lag time at any given temperature throughout most of the temperature range of interest based on the previous discussion.

It is highly impractical and expensive to conduct microbial spoilage tests for all possible temperature scenarios. A better approach is to conduct a limited number of experiments under constant temperature conditions to create a growth rate response surface and, then to use mathematical modeling techniques to estimate microbial spoilage for a given time-temperature history. It should be noted that this approach may not be effective or a significant error may exist if there is a time-temperature history effect for the growth. A history effect is one in which the actual growth rate that is measured after a temperature shift is significantly different from that predicted by a database model done at the same temperature. Several varying temperature studies of microbial growth showed some history effects [32,74,80,89], yet others did not find any history effect within the temperature range studied [59,93]. Another important factor that needs to be considered is the potentially different temperature-dependent behavior of different strains of the same species as well as different competitive species when exposed to a variable temperature sequence and the effects of those temperature changes on other hurdles. In addition to the above, studies at different water activities, oxygen levels and carbon dioxide levels to determine their singular and combined effects on growth rate would be needed.

Time-temperature integration

The use of mathematical models to study microbial growth under the fluctuating temperature conditions that prevail in commercial food distribution was first approached by Powers et al. [80] who predicted growth rate by multiplying the growth rate at the mean temperature by a fluctuating coefficient which was derived from a Q_{10} value. However, the actual and the predicted rates did not correlate well. The inaccuracy was attributed to the variation in Q_{10} which remains constant only for a relatively narrow temperature range and, to the average growth rate data which in this case combined both the lag and exponential phase. As noted previously, these usually do not change to the same extent with temperature change because of different activation energies.

Predicting the shelf-life of a food system with several different components becomes a problem when there are multiple modes of deterioration and each mode has its own temperature sensitivity [51]. The shelf-life of the whole product at any given temperature is determined by the mechanism in any component which proceeds fastest and thus causes the shortest life. Each food's metabolic pathway and natural flora are affected differently by temperature changes, a_w and gas composition dependence, which creates a real problem in terms of the ability to make shelf-life predictions.

Given that everything but temperature remains constant, one can simply use the following equation to integrate and determine the effect of a time-temperature history:

$$f(A) = \Sigma k_{[f(T_t)]} \mathrm{d}t \tag{12}$$

where $f(A)$ = quality function = ln N/N_o for first order microbial growth, $k_{[f(T_t)]}$ = time/temperature dependence of quality loss rate or growth rate of microbes. Taoukis and Labuza [96,97] have developed the method to solve this equation using the Arrhenius relation, which is an exponential function, and does not have an exact analytical solution. To simplify the solution, one divides the time-temperature exposure of the food into short, assumed-constant temperature intervals and then sums up the products on the right hand side as seen in Eqn 13. In fact any temperature model can be used to get the value of k_i for that segment.

$$\ln (N/N_o) = \sum_{i=1}^{i=n} k_i \, \Delta t_i \tag{13}$$

Obviously, the final level of the growth is dependent upon the initial number, which in most cases will be unknown, so a safe estimate is needed for the average initial bacterial load.

Recently, Van Impe et al. [102] proposed a dynamic model describing a bacterial population as a function of

both time and temperature, over the whole biokinetic temperature range of growth and inactivation. The model seems to be able to deal with time-varying temperature condition, but has not been validated in practice.

A good model must also encompass the growth rate-temperature response of all the species in the spoilage flora. It can be assumed that there will be considerable differences in these responses. Only a fraction of the initial flora will grow at chill temperatures. As the storage temperature rises, the fraction of the flora which grows will also increase; so the basic numbers as well as the growth rates are changing with temperature, and if there is competition or interaction, it is unlikely that any simple equation could model such a complex situation in a real food but that remains to be tested. Temperature-function integration may, therefore, have very restricted uses for spoilage or pathogen prediction, especially for CAP/MAP foods and only when good data are available [56].

Individual packages of a case or in a pallet load may experience a series of different environments corresponding to different locations during distribution and storage. Other packages from the same consignment will meet conditions which differ both in duration of storage and by traversing different parts of the distribution system. Furthermore packages are not identical, even if nominally the same, and will respond rather differently. Thus, one cannot assume that all packages will be stored for the same period under identical conditions, or will react in the same way.

An approach to solve for the temperature variation in a pallet would be to use a finite difference or finite element solution of the heat transfer equations [93], which facilitates the inclusion of: (i) variable environmental conditions; (ii) non-uniform initial conditions of temperature and bacterial load; (iii) temperature-dependent thermophysical properties of foods; and (iv) the thermal properties of the packaging material. An application of this for the growth of *Brochothrix thermosphacta* under fluctuating temperature conditions was illustrated by Simpson et al. [93]. In many cases one can assume that most of the damaging microbial growth will occur on the food surface, and that the surface closely follows the environmental conditions, thus a simple analytical equation can be employed. However, the temperature at the surface is affected by the thermal inertia of the entire food contents. For example, it has been shown that serving size may affect the level of temperature abuse [2].

Another approach to variable temperature shelf-life prediction was the graphical method developed by Charm et al. [15], who correlated spoilage of fish at different temperatures to the number of days of shelf-life at ice storage. From this they developed the 'shelf-life prediction slide rule' [86], which by a series of simple operations, allowed a rapid estimate of the remaining shelf-life of lean fish. It is essentially based on the log shelf-life model. Castell-Perez et al. [11] utilized a computer stimulation of the Monod equation for predicting microbial growth during freezing and the equilibration to frozen storage conditions. Thompson et al. [99,100] and Willardsen et al. [105] studied microbial dynamics under constant and rising temperatures during cooking of beef and showed good predictions. As seen, the number of actual tests of the second approach of predictive microbiology is very small but promising, however no one has attacked and proven the ability to predict under conditions where a_w and gas composition can change as well.

Time-temperature integrators

A time-temperature integrator/indicator is a device or tag that can keep track of an accumulated time-temperature distribution function to which a perishable product is subjected from the point of manufacture to the display shelf of the retail outlet, or even to the consumer [98]. The operation of a TTI is based on mechanical, chemical or enzymatic systems that change irreversibly from the time of their activation. The change is usually expressed as a visible response in the form of a mechanical deformation, color development and color movement. The rate constant for a TTI response usually follows the Arrhenius theory. The $E_{A(TTI)}$ values of the indicators cover the range of the most important deteriorative reactions in foods [30].

A kinetically based correlation scheme has been developed by Taoukis and Labuza [96] to allow prediction of the shelf-life of a product based on the TTI response. From the measured response of the tag and the tag kinetics, one can predict an effective temperature for a variable time-temperature distribution. This value is then used, with the equations for microbial growth kinetics to predict the amount of growth. This scheme assumes that the effective temperature response of the tag is equal to that of the food which is only true if the activation energies of the food and tag are equal [98]. Thus there is a need to have a tag with a temperature sensitivity close to that of the microbial growth resulting in quality loss. Fu et al. [33] designed such a tag theoretically by employing simultaneous diffusion and reaction. It should be noted that other models can also be applied as long as the same model is used to model both the TTI kinetics and the microbial growth kinetics.

Examples of TTI applications are given in detail by Taoukis et al. [98]. Potential use of time-temperature indicators as indicators of temperature abuse of MAP products has also been discussed [31]. A TTI can also be designed to be set to indicate a safe condition of a food product [56]. The applicability of single end point, consumer readable TTIs as monitors of the end of shelf-life of refrigerated food products was examined by Sherlock et al. [90]. Consumer TTIs can be reliably used as end of shelf-life indicators for foods with similar activation energy for deterioration (± 2 kcal mol^{-1}) [90]. One of the limitations of using these tags is that they react to package surface temperature and not the temperature of food content in a package, which, as pointed out earlier, may not respond to the environmental temperature fluctuation instantly. This would result in the unnecessary disposal of safe food with acceptable organoleptic quality [63].

Other approaches are to have electronic time-temperature recorders and integrate one of the predictive models in the hardware or download the data to a software package for

analysis on a computer. Daud et al. [22] predicted the remaining shelf-life of poultry tissues by incorporating the general spoilage curve of Olley and Ratkowsky [76,77] into an instrument called the temperature function integrator. This electronic-integrator device, developed by Nixon [75], to assess storage conditions in fish-holds, can give a readout of equivalent days of spoilage at 0 °C, and predict the remaining shelf-life of the product at 0 °C. There was close agreement between the relative growth rates of spoilage bacteria in poultry predicted by the integrator with experimental values at storage temperatures up to 16 °C. Above this temperature, significant deviations between the two values occurred. The value of this approach is that it eliminates the error of a TTI which may not have the same temperature response as the food.

A versatile time-temperature function integrator (TTFI) for predicting the degree of bacterial spoilage occurring in chilled foods during storage was described by Owen and Nesbitt [79]. The sensor of the TTFI (a thermistor) is attached by a flexible cable to the circuitry and display unit. The circuitry converts impulses received from the sensor, sums the temperature history and displays the integrated information as an equivalent number of days at a reference temperature. The remaining shelf-life may be calculated simply by subtraction of this value, from the expected shelf-life determined by storage trials at the reference temperature, or any other temperature in the psychrotrophic range.

Alternatives to monitoring temperature during food distribution include the use of flexible, miniaturized electronic temperature recording devices [60]. They record time-temperature information that can be displayed and processed at the receiving end by interfacing with a microcomputer. Recently a satellite tracking system (Geostar Satellite Tracking Service, Geostar Corp., Washington, DC) was introduced [55]. McMeekin and Olley [66] gave a list of other time-temperature recording systems. The key to all these systems is the use of the proper equations for modeling of growth and an understanding of kinetics. A common mistake is that simple integration of the area under a time-temperature curve is equivalent to shelf-life loss. One can quickly show that for a Q_{10} of 2 and an initial growth rate constant of 1 day^{-1} at 0 °C, storage for 20 days at 1 °C would give a count increase of e^{20} while storage at 20 °C for 1 day would give a count increase of e^{4}, a difference of e^{16} or 9 million CFU unit $weight^{-1}$. The areas under the time-temperature plot are equal but the area does not correlate with shelf-life.

FINAL THOUGHTS

Mathematical growth models have great potential for food microbiologists, giving them the most cost-effective means of predicting the microbiological stability and safety of our foods and combining the power of modern computers with the skill of microbiologists. Given a food with a large enough database and knowledge of the physical and chemical conditions, such as head space, gas composition, water activity and storage temperature, the microbial growth response can be predicted within the limits of that database. Once models are available for all microbes of concern, new formulations or new storage conditions could be evaluated without the need for slow, expensive microbiological tests [36]. Large databases are now under development to fill this need. A caution is that extrapolation of the models beyond the experimental limits may result in misleading predictions of microbial growth. Care should then be taken in the application of these models to variable conditions. First, it is important to ensure that the growth rate constant (or generation time) and the lag time are the key kinetic parameters to be modeled for shelf-life prediction. Secondly, good quality data are required to derive the models, so as to minimize the error in the prediction. Several researchers [38–40] have noted the discrepancies in the predictions based on their own data when compared to other published data. All conclude that this is most probably due to variations in the microorganisms, the growth medium or the storage conditions. In addition, all models generated should be validated, preferably by other workers, to ensure their suitability, robustness and application before general use. Almonacid and Torres [2] presented detailed procedures to estimate the reliability of predictive microbiology models making extensive use of non-parametric statistical procedures. In many cases industrial users are interested in deciding which refrigerated foods production and handling options are more effective in preserving quality and safety. In general, these applications require a lesser degree of model accuracy and a cost-effective application of predictive microbiology [93].

Current growth rate models have been based solely on constant temperature studies and seem to be adequate to predict rates at any other constant temperature within the temperature range studied. What is lacking are studies at temperatures outside the range and especially studies under cycling temperatures and knowledge of any history effect. There is a need for indicators capable of flexible design so that their expiration, as affected by storage, temperature and time, can be accurately set to follow biochemical or physical kinetics slightly faster than the rate of growth of specific target pathogens or toxin production. Ultimately, these indicators will provide a safe warning mechanism for the food processor, retailer and consumer. The confidence in the safety of new applications of modified atmospheres for food preservation will surely be greater if the scientific basis underlying these processes has been thoroughly studied.

Good management practices relating to the raw material quality, sanitation, processing conditions and storage temperatures will ensure the maximum quality, microbiological safety and shelf-life. In the near future computer programs and expert systems will be applied in predicting shelf-life and relative safety of perishables. International cooperation will advance the field of predictive microbiology.

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