Journal of Industrial Microbiology, 12 (1993) 180–189
© 1993 Society for Industrial Microbiology 0169-4146/93/$09.00
Published by The Macmillan Press Ltd

Bělehrádek-type models

T. Ross

Dept. of Agricultural Science, University of Tasmania, G.P.O. Box 252C, Hobart 7001, Tasmania, Australia

(Received 5 October 1992)

Key words: Predictive microbiology; Square-root model; Bělehrádek; Growth modeling

SUMMARY

Square-root (or Ratkowsky) models are a special case of Bělehrádek's temperature rate-relationship first published in 1926 and widely used in several fields of biology. Bělehrádek-type models also describe microbial growth, and have been extended for use in food microbiology by the inclusion of terms for water activity and pH. The parameters of the square root-type models are defined and their determination described. Favorable features of square root-type models include parsimony, parameter estimation properties, and ease of use. Square root-type models have been developed for a number of organisms of concern to the food industry and have also been adopted for use in a number of electronic devices used in predictive microbiology. Criticisms of square root-type models are also considered.

HISTORY AND DEVELOPMENT

Models for the suboptimal temperature range

In 1926 Bělehrádek and Porodko independently published the same formula which described the duration (or rate) of biological reactions as functions of temperature [3]. The function was:

$$y = \frac{a}{x^b} \qquad (1)$$

where y = duration of reaction,
x = temperature in °C and
a,b = constants to be fitted.

The transformation

$$\log(y) = \log(a) - b\log(x)$$

shows that the parameter a represents the duration of the process at 1 °C, and that the parameter b is a coefficient describing the effect of temperature on the reaction duration.

Bělehrádek noted that the function often did not fit the data well at very low temperatures or at temperatures above the optimum for growth and subsequently published [3] a modified form of the equation which does not assume that the response time at 0 °C is infinite, or, to use his terminology, recognizes that the 'biological zero' of the reaction may be other than 0 °C. This equation was written:

$$y = \frac{a}{(t-\alpha)^b} \qquad (2)$$

where a, b and y have the same meaning as in Eqn 1,
t = temperature in °C and
α = difference between the 'biological zero' and 0 °C, or more simply, 'the biological zero'.

More than half a century later Ratkowsky et al. [31] published the following expression relating the rate of microbial growth processes to temperature:

$$\sqrt{r} = b'(T-T_0) \qquad (3)$$

where: r = rate of process,
T = temperature in Kelvin,
T_0 = a conceptual temperature of no metabolic significance but which is an intrinsic property of the organism and
b' = the regression coefficient.

The equation was suggested by Ohta and Hirahara [27] who found empirically that a plot of temperature-vs-the square root of the rate of nucleotide degradation in cool-stored carp muscle was nearly linear. Noting that the duration of a reaction (y) is the reciprocal of its rate (r), Eqn 3 is seen to be a special case of Eqn 2 in which the exponent, b, has the value 2 and $b' = \sqrt{\frac{1}{a}}$. The use of the Kelvin temperature scale was recommended to avoid possible confusion regarding the sign of T_0.

Ratkowsky et al. [31] developed Eqn 3, which has come

Correspondence to: T. Ross, Dept. of Agricultural Science, University of Tasmania, G.P.O. Box 252C, Hobart 7001, Tasmania, Australia.
Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

to be known as 'the square root model', without knowledge of Bělehrádek's work, yet Eqn 2 has been used in biology, and particularly aquatic zoology, for many years [33].

Bělehrádek did not have available to him the statistical knowledge and computing power we enjoy today and stated that the calculation of α of Eqn 2, analogous to T_0 of Eqn 3, was not a simple mathematical operation and that α could only be assessed graphically with an accuracy of 5–10 °C [4]. In practice, Bělehrádek fitted the parameters by constructing a log–log plot after estimating a value for a, i.e.

$$\log(y) = \log(a) - (b)\log(t-\alpha) \tag{4}$$

Ratkowsky (pers. comm.) found, upon examination of the residuals of bacterial growth rate data when fitted to Eqn 3, that the exponent 2 is, or very nearly is, the most appropriate value for this parameter. Furthermore, McMeekin et al. [21] using nonlinear regression fitted an extensive data set for the growth of *Staphylococcus xylosus* to Eqn 2. The exponent, b, was estimated to be 1.97 ± 0.04. These observations support the validity of the square root model which, because the exponent is a constant value, may be fitted in the form of Eqn 3 by simple linear regression (subject to stochastic considerations discussed later).

Ratkowsky et al. [31] showed: that Eqn 3 fitted 71 original data sets for the growth of bacterial populations, as well as 41 data sets obtained from the literature and other independent sources; that it also describes well the temperature/rate relationship of the deterioration of proteinaceous foods; and that it describes the growth rate response of several yeasts investigated. Equation 3 has been shown to describe the growth rate responses of many species of bacteria by other workers [5,13,19,37] and Bremner et al. [7] have shown that Eqn 3 describes organoleptic changes associated with the deterioration of seafood products.

Models for the full biokinetic temperature range

Ratkowsky et al. [30] presented an extension of Eqn 3 which enables it to model growth over the entire biokinetic range, that is, from the minimum to the maximum temperatures permissive for growth. This equation is:

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

where: r and T have the same meaning as in Eqn 3,

$T_{\min}$ = a lower theoretical temperature, analogous to T_0 of Eqn 3, at and below which no growth is possible,

$T_{\max}$ = an upper theoretical temperature at and above which no growth is possible and

b and c are parameters to be fitted.

Equation 5 is nonlinear, and requires more complex techniques to fit than does Eqn 3. Ratkowsky et al. [30] state that by fitting Eqn 5 to data an estimate of T_{opt}, the temperature at which the growth rate is maximal, can be obtained and that together with the paramters $T_{\min}$ and $T_{\max}$ these may be considered to be cardinal temperatures by which organisms may be classificed as psychrophiles, mesophiles or thermophiles more objectively.

Kohler et al. [16], Heitzer et al. [15] and Zwietering et al. [39] fitted data to a form of Eqn 5 in which both sides of the equation were squared so that the response variable is rate. From this modified form Zwietering et al. [39] developed another modified form of Eqn 5 in which the superoptimal component, $(1-\exp[c\{T-T_{\max}\}])$, is not squared. They showed that when Eqn 5 is expressed with rate as the dependent variable the decline in specific growth rate above the optimum temperature for growth is described better by an exponential function than the square of an exponential function. This second Zwietering et al. [39] model is written, using consistent nomenclature:

$$r = [b(T-T_{\min})]^2\,\{1-\exp[c(T-T_{\max})]\} \tag{6}$$

Most work in predictive microbiology which has relied on Bělehrádek-type models, has centered on the use of the simple square root model, Eqn 3, which is now usually written:

$$\sqrt{r} = b(T-T_{\min}) \tag{7}$$

where all parameters are as previously defined.

It is noteworthy that this model has been incorporated into several commercially available electronic devices developed for the prediction of product safety and remaining shelf-life on the basis of temperature history. The two devices that are currently marketed are the Remonsys Smartlog (Remonsys Ltd, Unity Rd, Bristol BS18 1NH, UK) and the Delphi Temperature Logger (Management Information Resources Ltd, PO Box 3680, Wellington, New Zealand). The Smartlog is a temperature logger which, in addition, features on-board processing of the temperature history to provide an indication of the remaining shelf-life of the product relative to that at some preferred storage temperature. The estimate of the elapsed shelf-life is based on the concept of relative rates, discussed later, and is calculated using Eqn 7. The Delphi Logger is a compact and robust temperature logger originally designed for New Zealand's export meat industry. The logger itself is not extraordinary but the accompanying software enables estimates of the extent of growth of *E. coli* under various processing and packaging scenarios to be made based upon the downloaded temperature history of the product. The *E. coli* growth data is modeled by a modified form of Eqn 7. A system like the Delphi could easily be extended by the inclusion of software incorporating models for other organisms of interest. A single temperature history could then be interpreted in terms of the potential growth of any organism for which a model was included.

Models incorporating the effect of other rate modifying factors

(i) Water activity. McMeekin et al. [21] presented a model

which describes the combined effects of water activity and temperature on growth and lag phase resolution rate for *Staphylococcus xylosus* in the sub-optimal temperature and water activity ranges. A general form of this model was proposed by Chandler and McMeekin [9] which may be written:

$$\sqrt{r} = b\sqrt{(a_w - a_{w_{min}})}\,(T - T_{min}) \tag{8}$$

where: r, T, and T_{min} are as previously defined,
b = a regression coefficient,
a_w = water activity and
$a_{w_{min}}$ = a theoretical water activity at and below which the response time is infinite.

Equation 8 is a synthesis of Eqn 7 and the observed growth rate response of bacteria to a_w, which may be modeled by the expression:

$$rate = C(a_w - a_{w_{min}}) \tag{9}$$

where C is a regression coefficient. The growth rate response to water activity had been observed earlier [36]. McMeekin et al. [21] and Chandler and McMeekin [9,10] showed that T_{min} did not change with changing water activity or the humectant used, but that the $a_{w_{min}}$ was dependent upon the humectant.

The form of the equation is a reflection of the observation that the effects of temperature and water activity are additive, rather than synergistic. This observation is also apparent in Davey's Modified Arrhenius Model [11]. Where either temperature or water activity is constant Eqn 8 simplies readily into Eqn 9 or Eqn 7 respectively. Equation 8 has been successfully applied to the growth of other organisms. Ross and McMeekin [34] developed a model of the type of Eqn 8 for the growth of *Staphylococcus aureus* and Hayward [14] constructed models of the type of Eqn 8 for several strains of *Aeromonas hydrophila*.

(ii) pH. Adams et al. [2] used a similar experimental approach to that of McMeekin et al. [21] to develop models for the combined effects of pH and temperature on the growth of *Yersinia enterocolitica* for a variety of acidulants. The models which they developed have the general form:

$$\sqrt{r} = b''\sqrt{(pH - pH_{min})}\,(T - T_{min}) \tag{10}$$

where: r, T, T_{min} have the same meaning as above,
b'' = a regression coefficient
pH_{min} = a 'theoretical' pH at and below which the growth rate is 0 (analogous to $a_{w_{min}}$ in Eqn 8).

The parameter pH_{min} was found to be dependent upon the acidulant used but T_{min} was found to remain invariant, indicating that pH and temperature also act independently.

McMeekin et al. [24] have speculated on the possibility of a model of the square root type which encompasses the effects of temperature, water activity and pH and which might have the form:

$$\sqrt{r} = C\sqrt{(a_w - a_{w_{min}})}\,\sqrt{(pH - pH_{min})}\,(T - T_{min}) \tag{11}$$

ESTIMATION OF THE PARAMETER VALUES

Estimation of the values of the parameters of the simple square root model and its derivatives has been undertaken by a number of methods. The simple square root model, Eqn 7, may be fitted by simple linear regression which yields an equation of the form:

$$y = bx + c$$

where $x = T(\mathrm{K})$, $y = \sqrt{rate}$, b = the regression coefficient, and $-c/b = T_{min}$. Figure 1 depicts a typical square root plot and shows the interpretation of the parameters. Note that the parameter T_{min} is the intercept of the regression line through the data with the temperature axis, not the minimum temperature at which growth is observed. Similarly T_{max} is a notional temperature given by the intercept of the regression line with the temperature axis, and is not the same as the maximum temperature at which growth is observed. The parameter b is the slope of the regression line in the suboptimal region. The parameter c is a regression coefficient related to the rate of decrease of growth rate with increasing temperature above the optimum for growth. The Kelvin scale is advocated to avoid potential confusion regarding the sign of T_{min} [24].

Equations 5 and 6 must be estimated by nonlinear regression procedures which employ an iterative process and require the estimation of initial parameter values. It is an advantage of the square root-type models that they have readily interpretable parameters which aid this process. Methods for deriving initial parameter estimates of Eqn 5 are given by Ratkowsky [28] and are also appropriate for Eqn 6. Interpretation of the parameters of Eqn 5 are also shown in Fig. 1.

There is a strong temptation, when generating models of

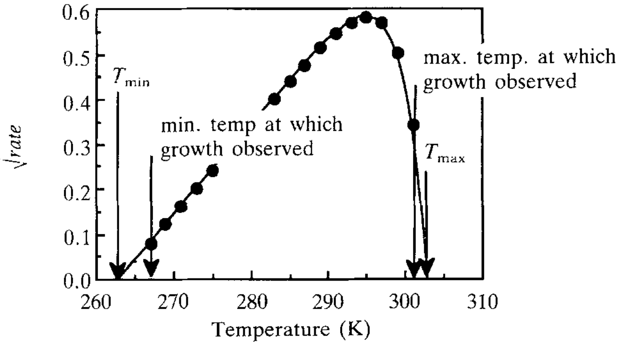

Fig. 1. A typical square root plot encompassing data throughout the biokinetic range, showing the interpretation of the parameters of Eqn 7.

the type of Eqn 8 or Eqn 10 to build the model sequentially, that is, to generate data from which to derive a value for the parameter $T_{\min}$, and then to generate data to derive a value for the second parameter, and so on. This methodology was employed [21,34] for Eqn 8, and for Eqn 10 [2] using simple linear regression, as shown in Fig. 2(a,b). McMeekin et al. [21] and Adams et al. [2] generated simple square root equations at many water activity levels and pH levels respectively. The parameter b of the square root equations changed systematically with changes in these variables. Linear regression of b^2-vs-rate was performed to determine $a_{w_{\min}}$ or $pH_{\min}$ respectively. From the data of McMeekin et al. [21] and Chandler and McMeekin [9], Ross and McMeekin [34] concluded that $T_{\min}$ was independent of water activity and estimated $a_{w_{\min}}$ by calculating a linear regression for water activity-vs-growth rate from data obtained isothermally. If it were demonstrated that these parameters ($T_{\min}$, $a_{w_{\min}}$, $pH_{\min}$) are invariant properties of the modeled organism for a given humectant or acidulant for example, then a rapid and efficient method for generating models can be envisaged. Equations 8 and 10, however, are nonlinear models and the rigorous method for developing models of this type is to fit all data simultaneously using nonlinear regression techniques. Nonetheless, given sufficient reliable data the two approaches would be expected to generate models with very similar parameter estimates.

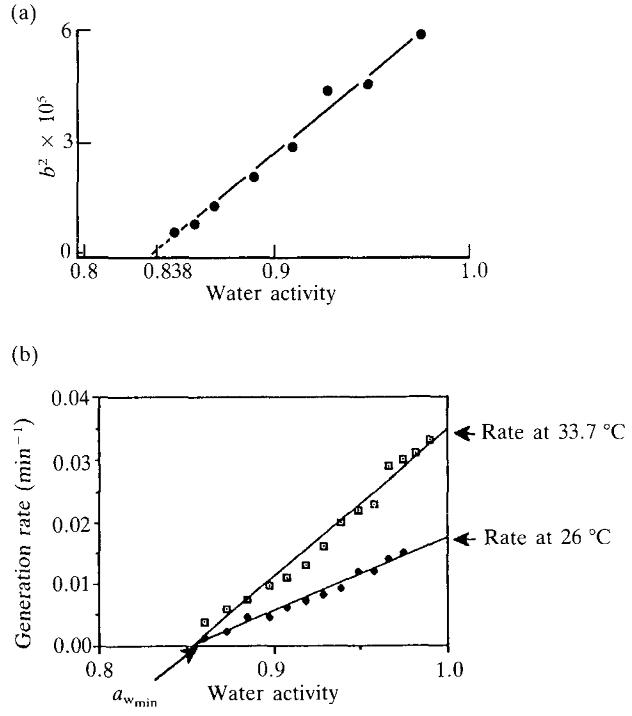

Fig. 2. Estimation of $a_{w_{\min}}$ and $pH_{\min}$, using $a_{w_{\min}}$ as an example. (a) shows the estimation method of McMeekin et al. [21] (reproduced from McMeekin et al. [21] with permission of Blackwell Scientific Publications). The method of Ross and McMeekin [34] is shown in (b) (reproduced from Ross and McMeekin [34], with permission of the Council of Australian Food Technology Associations Inc. and the Australian Institute of Food Science and Technology Ltd). n.b. Generation rate = generations per unit time.

It is apparent from the form of Eqns 8 and 10 that, were temperature held constant, these models would degenerate into simpler models such as:

$$\sqrt{r} = B'\sqrt{(a_w - a_{w_{\min}})} \tag{12a}$$

or

$$r = (B')^2\,(a_w - a_{w_{\min}}) \tag{12b}$$

and

$$\sqrt{r} = C'\sqrt{(pH - pH_{\min}} \tag{13a}$$

or

$$r = (C')^2\,(pH - pH_{\min}) \tag{13b}$$

Data may be fitted to these functions by simple linear regression expressed with rate as the dependent variable (Eqns 12b and 13b), obtained by squaring both sides of equations 12a and 13a, respectively. Such a transformation may be statistically invalid, however, as will be demonstrated below.

ADVANTAGES OF THE SQUARE ROOT MODELS

To most users of predictive models the most important question is: *how well does the model describe the data.* Of even greater importance is how accurately the model predicts, that is, how well it models observations not used to estimate the values of the parameters of the model. These characteristics of the model are intimately related to a number of other properties of models which are considered by Ratkowsky [29] who lists criteria which may be used to compare and evaluate competing models. His list includes:

appropriateness of the stochastic assumption,
parsimony,
parameter estimation properties,
interpretability of parameters and
range of variables.

Although an assessment of the square root models in relation to the above criteria will be considered here readers are referred to Ratkowsky [29] for a full discussion of their importance.

Goodness-of-fit

McLaren [20] ascribed the popularity of Bělehrádek-type models to the fact that they have few parameters, i.e. they are parsimonious, and that they fit the data well. In relation to the square root-type models, comparisons which have been undertaken [15,32,39] suggest that the square root model and its various derivatives describe bacterial growth responses to temperature *at least* as well as the other models that have been advocated for use in predictive microbiology.

Li [17] applied Eqn 5 to the description of growth rates of phytoplankton assemblages and concluded that 'the Ratkowsky model not only fits the data well but has parameters that are easily identified with biological concepts'.

It is important to recognize that goodness-of-fit of a model to a specific data set does not of necessity translate to predictive ability. The most important feature of a *predictive* model is that it accurately models the general response. The more parameters that a model has the more closely it will be able to model a specific data set, but if that data set is not representative of the normal response its predictions may be less accurate because the model may lack universality [11]. It is a sterile exercise to compare goodness-of-fit unless one is confident that the data used for the comparison are representative of the typical response. In practice replicated data are required, and particularly where the response is very variable such as when the organism is growing near to the limit of its growth range. These considerations are emphasized by the comparison of models undertaken by Zwietering et al. [39] who distinguished variability within the data from lack of fit.

Predictive ability

Several published reports deal with the predictive ability of square root-type models. Chandler and McMeekin [8] developed and validated a square root-type model for the psychotrophic spoilage of milk. Smith [38] found that predictions from his previously derived model [37] could be used to estimate the increase in coliforms, *E. coli* and salmonellae on meat allowed to warm again after overnight chilling. In most cases the predictions were within one 'population doubling' of the observed results. Ross and McMeekin [34] derived a model of the type of Eqn 8 for the growth rate of *Staphylococcus aureus* in response to temperature and water activity (NaCl as humectant). Though the data were generated from optical density measurements in laboratory broths, the model predicted well the growth of the same organism on seafood products under 17 different conditions of water activity and temperature determined by viable count methods. The numerous publications and industry application of the results of work performed at the Meat Industry Research Institute of New Zealand, under the direction of C.O. Gill, are further examples.

Parsimony

It is desirable to be able to differentiate the true general response underlying the observed data from the trends in specific data sets. As stated above, models with many parameters may lack universality and in this regard parsimonious models are likely to be more robust because they have fewer parameters with which to accommodate unusual points. The square root-type models are the most parsimonious of any of the models presented in the literature to date, but achieve parsimony without sacrificing goodness-of-fit [39]. One might infer from this that the square root-type models more accurately model the true response, i.e. the response which is most representative of the typical behavior.

Parameter interpretability

The parameters of the square root-type models, although having no certain *physiological* interpretation, are easily and clearly defined allowing the effect of individual factors to be easily assessed, and allowing for simplification of the expression if one independent variable is constant (see Eqns 12a and 13a). Li [17], however, discusses possible biological interpretations of the parameters of Eqn 5 and Ross [33] reviewed discussion concerning the parameters of Eqn 2. Parameter interpretability also aids in the derivation of initial parameter estimates for nonlinear regression procedures.

Parameter estimation behavior

The square root-type models display close to linear behavior which leads to good parameter estimation properties [16].

Stochastic assumptions

Ratkowsky et al. [32] analyzed the behavior of the variability of bacterial growth rate estimates. The importance of this behavior is that it dictates how growth rate data should be manipulated to yield reliable predictive models and provides information about the confidence one can have that an observation is representative of the 'usual' response.

Using the data of Smith [37] Ratkowsky et al. [32] showed that, for *E. coli* the variance in growth rate is a function of the rate itself, that is, the faster the rate, the greater the variance in rate. The implication of this is that when models are fitted to data by the method of least squares, as they commonly are, those data having larger magnitude will have more influence in the fitting process. This is because the sum of the squares will be minimized by fitting more closely to those points as was demonstrated clearly by McMeekin et al. [23].

It is crucial when fitting data by the method of least squares that a transformation of the data is found in which the variance has the same magnitude irrespective of the magnitude of the response, or alternatively that a suitable weighting is applied to the data when fitting. The data of Smith [37] for the growth rate response of *E. coli* showed that variance is homogeneous in the square root of rate, as shown in Fig. 3. The data of Neumeyer [26] for the growth of *Staphylococcus aureus* support this observation. Currently, there is insufficient published data to conclude whether one transformation of the response variable is appropriate in all cases. A more general approach to modeling kinetic data has now been advocated by McMeekin et al. [22], who recommend that, before modeling, the data be examined to reveal the stochastic behavior. A transformation appropriate to that behavior should then be applied to the data to give all points equal weight in the fitting process.

The consequences of the stochastic assumption

Ratkowsky et al. [32], as well as showing that for *E. coli* the variance in bacterial growth rate is homogeneous in the square root of growth rate, also demonstrated the consequence of this for the prediction of the time for bacterial growth processes, such as spoilage or resolution of

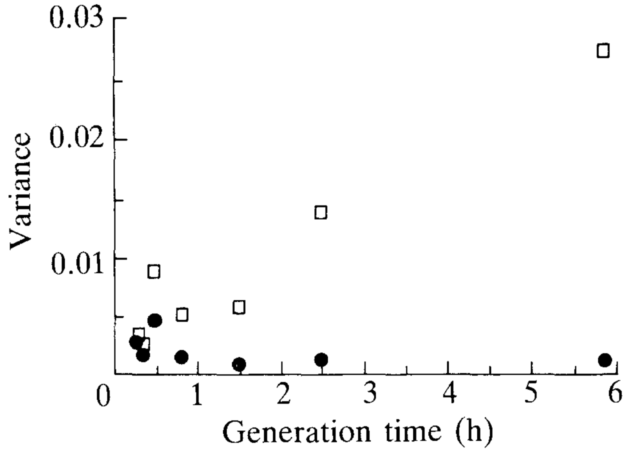

Fig. 3. Comparison of the variance in $\sqrt{rate}$ and the variance in ln *rate* for the generation time data of Smith (pers. comm). ●: Var $\sqrt{rate}$; □: Var (ln *rate*). (Reproduced from Ratkowsky et al. [32] with permission of Blackwell Scientific Publications.)

the lag phase, to occur. They showed that the variance in time, Var(ϕ), is related to the predicted time, ϕ, and the variance in the square root of rate, Var($\sqrt{r}$), by the equation:

$$\mathrm{Var}(\phi) = 4\,\phi^3\,\mathrm{Var}(\sqrt{r}) \tag{14}$$

that is, the confidence intervals of the predicted response time widen enormously as the predicted duration of the process increases. By using the square root transformation and Eqn 14 the variance calculated at one temperature can be used to estimate the variance in a predicted response *time* at any other temperature. In view of the increasing variability of growth rate estimates under severe growth limiting conditions completely accurate predictions cannot be made from limited data sets. The significance of the constancy of the error in the $\sqrt{rate}$ transformation is that by using Eqn 14 one can estimate, from the variance of data obtained more easily under less severe growth conditions, the variance in growth rate under any other conditions. When reliable estimates of the mean response and variance are available it will be possible not only to predict a growth response but to state the degree of confidence in that prediction, and from this to make objective assessments of the quality and safety of foods [32].

Ease of use

That the square root-type models are parsimonious suggests that they are also easier to work with on a day-to-day basis than some other model types proposed. One example of this is the relative rate concept which can be applied to any product of consistent starting quality and of known shelf-life, provided that the T_{min} of the dominant spoilage microbiota is known. For many proteinaceous foods of high water activity the dominant spoilers are pseudomonads which typically have a T_{min} of −10 °C. From this one can derive an expression for the relative rate of spoilage at any temperature relative to that at the preferred storage temperature.

$$\text{Relative Rate} = \frac{\text{Rate at actual temperature}}{\text{Rate at preferred temperature}}$$

$$= \frac{b(T - T_{\mathrm{min}})^2}{b(T_{\mathrm{ref}} - T_{\mathrm{min}})^2}$$

$$= \frac{(T - T_{\mathrm{min}})^2}{(T_{\mathrm{ref}} - T_{\mathrm{min}})^2}$$

where T_{ref} = preferred storage temperature

which for meat products, with a recommended storage temperature of 0 °C simplifies to: Relative Rate = $(0.1\,t + 1)^2$ where t is in °C. This relationship forms the basis of the Temperature History Index calculated by the Smartlog temperature history integrator and logger. By analogy similar expressions could be developed for any of the variants of the square root model. The relative rate function is easier to determine using square root-type models because the respective terms are multiplicative and, as a consequence, the expression simplifies readily.

Combined lag and exponential growth models

Lag phase resolution and exponential growth appear to have the same T_{min} [9,10,21,26] which, provided that the lag time is reproducible, enables a single model to be developed for the time taken for a population to achieve a particular level of growth including resolution of the lag phase. This property is of particular use when predicting shelf-life because a single model may be used rather than separate models for lag resolution and subsequent growth of the population to spoilage levels.

Implications for experimental design

As alluded to previously the observations that temperature and water activity, and temperature and pH act independently have particular advantages for a model of the form of the modified square root models, Eqns 8 and 10, because it suggests that the parameters T_{min}, pH_{min} and $a_{\mathrm{w}_{\mathrm{min}}}$ may be considered to be constants. Thus simple experiments could be performed independently to derive accurate values for these parameters, which could then be substituted into the equations.

This approach would enormously reduce the workload required to accumulate the data normally considered necessary to build reliable predictive models for food microbiology. For example, if one were interested in the growth of *Salmonella* in food in which water activity was modified by NaCl a model of the type of Eqn 8 could be constructed by performing two experiments. The first, at constant a_{w} and multiple temperatures would enable determination of T_{min}; the second at constant temperature and multiple a_{w} would enable determination of $a_{\mathrm{w}_{\mathrm{min}}}$. The parameter b can be evaluated by substitution. It is tempting to speculate that a three-variable model, such as Eqn 11, might be constructed analogously by performing three simple experiments. It must be remembered, however, that at this time the parameters T_{min}, $a_{\mathrm{w}_{\mathrm{min}}}$ and pH_{min} are statistical estimates of characteristics that by definition cannot be measured directly, and that consideration would have to be given to the amount and range of data used to derive those parameter values. Currently there are insufficient data on the combined effects of these three variables and whether they all act independently. In addition one would have to perform replicated experiments under several sets of conditions to enable determination of the variance in $\sqrt{r}$.

CRITICISMS OF THE SQUARE ROOT MODEL

Several criticisms of the square root model have been expressed and are considered below.

Poor fit to data

The most direct criticism was that of Adair et al. [1]. Their conclusions were criticized [15,23,32,39] because their method of comparison did not take into account that mathematical transformation of data results in a different weighting of different numerical values, i.e. the stochastic assumptions inherent in their comparison were unsound.

T_{min} *does not represent the minimum growth temperature*

$T_{\min}$ was defined earlier and it has been emphasized that this parameter does not represent the minimum temperature at which growth is measurable. It has been observed [9,10, 21] that as environmental conditions become more severe the rate of growth slows and the minimum temperature at which growth is observed increases. A possible interpretation of this may be found by considering a simple model of growth in which, under constant conditions, the cell requires a fixed amount of energy to maintain basal functions and directs any surplus energy towards reproduction. As conditions become less favorable for growth, for example due to lower water activity, the cell requires more energy for basal functions such as production of compatible solutes, active transport of ions, etc. and consequently has less available for reproduction. Consequently it takes longer to accumulate the surplus energy required to replicate, perceived as a slower growth rate. We may also apply thermodynamics to the interpretation of the minimal temperature for growth. Using the reconciliation of the Arrhenius Law and Eqn 7 [21]:

$$E = \frac{2RT^2}{T - T_{\min}} \qquad (15)$$

to estimate the activation energy (E) for growth from square root model parameters, it is apparent that an increase in the minimum temperature at which growth is possible may be interpreted as an increase in the energy barrier to growth.

Continuing this paradigm, it is interesting to speculate whether as temperature falls the metabolic rate of maintenance functions declines towards the same $T_{\min}$ as the growth rate response. This possibility could be investigated by, for example, determining the response to temperature of metabolic rate using as an index the rate of CO_2 evolution.

Lack of fit at low temperatures

Anecdotal evidence has suggested that the simple square root model shows a systematic lack of fit at low temperatures, i.e. near $T_{\min}$. A number of explanations have been suggested. One is that when a cocktail of organisms is used to generate data the individual strains may be expected to have different $T_{\min}$ and b values. It can be shown both mathematically [22] and practically [14] that the consequence of this is that the resultant square root plot is similar to that which would be obtained if the individual square root plots were superimposed, as shown in Fig. 4, which shows that although the response of each organism obeys the square root relationship, the response of the population is a combination of the individual responses. This occurs because the population growth rate at any temperature will tend toward that of the strain which grows fastest at that temperature, as that strain outgrows the other.

Another explanation is that when collecting data one may expect variation in the rates observed and that there is a systematic bias against the collection of data representative of slower growing cultures, i.e. those data which would fall below the regression line, because the experiment is terminated before such data are collected.

An empirical model

The second line of criticism, though not explicitly expressed, seems to be mistrust of empirical models. Evidence for this may be found in Heitzer et al. [15] who concluded that the square root model had equal utility to the Schoolfield et al. [35] model 'even though the square root model is devoid of any conceptual basis.'

Draper [12] discusses the relative merits of empirical and mechanistic models. He considers mechanistic models preferable because:

(i) they usually contain fewer parameters,
(ii) they usually fit the data better and
(iii) they usually extrapolate more sensibly.

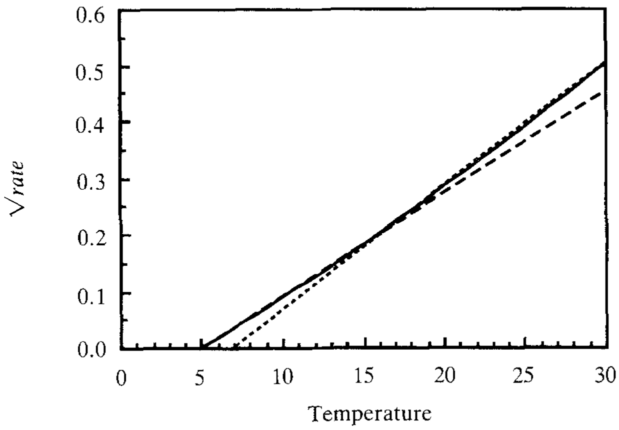

Fig. 4. A diagram showing the effect on the observed growth rate response to temperature of a mixture of two strains/species (assuming that each strain is initially present in equal proportion).

Of the models currently advocated for use in predictive microbiology only the master reaction-type models, of which the Schoolfield et al. model [35] has received most attention, purport to have a theoretical basis. With regard to Draper's first two reasons for preference, the Schoolfield et al. model has more parameters than analogous square root-type models and it does not consistently better fit the data. On Draper's third basis for preference, Lowry and Ratkowsky [18] showed that the Schoolfield et al. model predicts zero growth rates only at infinitely high or infinitely low temperatures. They concluded that the model was incomplete as it failed to take into account irreversible enzyme reactions. The inability to predict zero growth rates at other than infinitely high or low temperatures is a characteristic of all Arrhenius-type models proposed for use in predictive microbiology, at the time of writing. It has been shown [23] that the Schoolfield et al. model may produce unrealistic if not impossible estimates of its thermodynamic parameters, and Heitzer et al. [15] concluded of the thermodynamic constants of the master reaction-type models that they 'can no longer be regarded as true thermodynamic properties unless a single growth rate determining reaction can be identified. As this is usually not the case . . .'. One is drawn to the conclusion that none of the models currently advocated for use in predictive microbiology can be considered truly to be mechanistic.

Olley (pers. comm.) has reviewed the contemporary literature on conformational thermodynamics of proteins and concluded that the Schoolfield et al. model is inadequate to describe the effect of temperature on enzyme activity because it does not take into account the effect of heat capacity changes in the enzyme which are a function of temperature and which contribute to the Gibbs free energy of reversible denaturation. Brandts [6] developed a model similar in concept to the Schoolfield-type models, but which incorporated the effect of heat capacity changes. Brandts was unable to mechanistically model the heat capacity changes as a function of temperature, and used an empirical expression to describe the response. Murphy et al. [25] presented a mechanistic model for the Gibbs free energy of reversible protein denaturation which includes the effect of temperature on the heat capacity of proteins.

Olley and Ross (unpublished) have combined the model of Murphy et al. [25] with the Brandts model [6] to yield the following master reaction-type model:

$$rate = \frac{C\exp(-\Delta E_a/RT)}{1+\exp(-n(\Delta H^* - T\Delta S^* + \Delta C_p \\ [\{T - T^*_H\} - T\ln\{T/T^*_S\}])/RT)} \tag{16}$$

where C = a parameter whose value must be estimated,

ΔE_a = activation energy of the reaction catalyzed by the rate-limiting enzyme,

ΔC_p = difference in heat capacity between the N- and D-state of the rate-limiting enzyme,

n = number of amino acid residues in the rate-limiting enzyme,

T^*_H = temperature (K) at which the ΔC_p contribution to enthalpy is 0, a constant,

T^*_S = temperature (K) at which the ΔC_p contribution to entropy is 0, a constant,

ΔH^* = value of enthalpy (per mol amino acid residue) at T^*_H,

ΔS^* = value of entropy (per mol amino acid residue) at T^*_S,

T = temperature (K) and

R = gas constant (8.314 J K^{-1} mol^{-1}).

Using parameter values based on representative literature values this model generates data that are consistent both with a thermodynamic treatment of bacterial growth kinetics and with a square root-type model as shown in Fig. 5. The goodness-of-fit of the square root model to the data suggest that a convergence between empirical and mechanistic approaches may ultimately be achieved. This line of investigation, however, is quite speculative.

CONCLUSIONS

Square root-type models have proven to be useful in predictive microbiology as well as other fields of biology. They fit the data well; are linear or close to linear and consequently have good parameter estimation properties; have interpretable parameters; are appropriate to the stochastic properties of bacterial growth rates and are easy to use. They have found acceptance by a wide range of workers and have been incorporated into commercial devices for monitoring the microbiological shelf-life and safety of foods.

Published criticisms of square root-type models have been shown to be unfounded or inappropriate. Rigorous critical

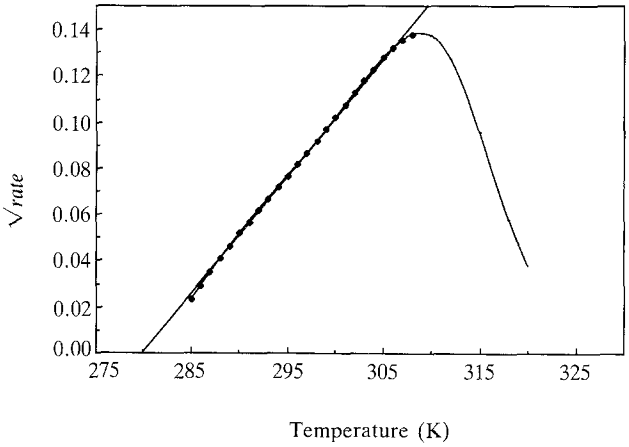

Fig. 5. Data generated by the Brandts/Murphy model (Eqn 15), and presented as a square-root plot. The parameters were assigned the following values: ΔE_a = 80000 J mol^{-1}; $C = 1 \times 10^{12}$ (unit volume–time)$^{-1}$; ΔC_p = 75 J K^{-1} (mol–amino acid residue)$^{-1}$; ΔH^* = 4655 J (mol–amino acid residue)$^{-1}$; $\Delta S^* \cdot 18.9$ J K^{-1} (mol–amino-acid residue)$^{-1}$; T^*_s, T^*_H = 385 K; and the rate-controlling enzyme has 300 amino acids residues. Linear regression of the predicted rate values at 1-K intervals (◆), over the range 285–3008 K, was performed. The fitted line (r^2 = 0.999) is shown.

comparisons that have been undertaken suggest that square root-type models perform at least as well as any other kinetic models advocated for use in predictive microbiology.

ACKNOWLEDGEMENTS

The author is supported by a stipend from the Rural Industries Research and Development Corporation, Australia. Additional travel funds were provided by the Australian Department of Industry, Trade and Commerce. The assistance of both organizations is gratefully acknowledged.

REFERENCES

1 Adair, C., D.C. Kilsby and P.T. Whittall. 1989. Comparison of the Schoolfield (non-linear Arrhenius) model and the Square Root model for predicting bacterial growth in foods. Food Microbiol. 6: 7–18.

2 Adams, M.R., C.L. Little and M.C. Easter. 1991. Modeling the effect of pH, acidulant and temperature on the growth rate of *Yersinia enterocolitica*. J. Appl. Bacteriol. 71: 65–71.

3 Bĕlehrádek, J. 1930. Temperature coefficients in biology. Biol. Rev. Biol. Proc. Camb. Philos. Soc. 5: 30–60.

4 Bĕlehrádek, J. 1954. Temperature and rate of enzyme action. Nature (Lond.) 173: 70–71.

5 Blankenship, L.C., S.E. Craven, R.G. Leffler and C. Custer. 1988. Growth of *Clostridium perfringens* in cooked chili during cooling. Appl. Environ. Microbiol. 54: 1104–1108.

6 Brandts, J.F. 1964. The thermodynamics of protein denaturation. II. A model of reversible denaturation and interpretations regarding the stability of chymotrypsinogen. J. Am. Chem. Soc. 86: 4302–4314.

7 Bremner, H.A., J. Olley and A.M.A. Vail. 1987. Estimating time-temperature effects by a rapid systematic sensory method. In: Seafood Quality Determination (Kramer, D.E. and J. Liston, eds), pp. 413–435, Elsevier Science Publishers, Amsterdam.

8 Chandler, R.E. and T.A. McMeekin. 1985. Temperature function integration and the prediction of the shelf-life of milk. Aust. J. Dairy Technol. 40: 10–13.

9 Chandler, R.E. and T.A. McMeekin. 1989a. Modeling the growth response of *Staphylococcus xylosus* to changes in temperature and glycerol concentration/water activity. J. Appl. Bacteriol. 66: 543–548.

10 Chandler, R.E. and T.A. McMeekin. 1989b. Combined effect of temperature and salt concentration/water activity on the growth rate of *Halobacterium* spp. J. Appl. Bacteriol. 67: 71–76.

11 Davey, K.R. 1989. A predictive model for combined temperature and water activity on microbial growth during the growth phase. J. Appl. Bacteriol. 67: 483–488.

12 Draper, N.R. 1988. Response surface designs. In: Encylopaedia of Statistical Sciences, Vol. 8 (Kotz, S. and N.L. Johnson, eds), pp. 107–119, Wiley-Interscience, John Wiley and Sons, New York.

13 Griffiths, M.W. and J.D. Phillips. 1988. Prediction of the shelf life of pasteurized milk at different temperatures. J. Appl. Bacteriol. 65: 269–278.

14 Hayward, L.J. 1990. Predictive microbiology of *Aeromonas hydrophila*: the effect of temperature and water activity on the growth rate of *Aeromonas hydrophila*. BSc (Hons) Thesis, University of Tasmania.

15 Heitzer, A., H.-P. E. Kohler, P. Reichert and G. Hamer. 1991. Utility of phenomenological models for describing temperature dependence of bacterial growth. Appl. Environ. Microbiol. 57: 2656–2665.

16 Kohler, H.-P.E., A. Heitzer and G. Hamer. 1991. Improved unstructured model describing temperature dependence of bacterial maximum specific growth rates. In: International Symposium on Environmental Biotechnology (Verachtert, H. and W. Verstraete, eds), pp. 511–514, Royal Flemish Society of Engineers, Ostend, Belgium.

17 Li, W.K.W. 1985. Photosynthetic response to temperature of marine phytoplankton along a latitudinal gradient (16°N to 74°N). Deep-Sea Res. 32: 1381–1391.

18 Lowry, R.K. and D.A. Ratkowsky. 1983. A note on models for poikilotherm development. J. Theor. Biol. 105: 453–459.

19 Mackey, B.A. and A.L. Kerridge. 1988. The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef. Int. J. Food Microbiol. 6: 57–65.

20 McLaren, I. 1966. Temperature and frogs eggs: a reconsideration of metabolic control. J. Gen. Physiol. 48: 1071–1079.

21 McMeekin, T.A., R.E. Chandler, P.E. Doe, C.D. Garland, J. Olley, S. Putro and D.A. Ratkowsky. 1987. Model for combined effect of temperature and salt concentration/water activity on the growth rate of *Staphylococcus xylosus*. J. Appl. Bacteriol. 62: 543–550.

22 McMeekin, T.A., J. Olley, T. Ross and D.A. Ratkowsky. 1993. Predictive Microbiology: Theory and Applications. Research Studies Press, Ltd, Somerset, England, John Wiley and Sons, Inc.

23 McMeekin, T.A., D.A. Ratkowsky, J. Olley and T. Ross. 1989. Comparison of the Schoolfield (non-linear Arrhenius) model and the Square Root model for predicting bacterial growth in foods – a reply to C. Adair et al. Food Microbiol. 6: 304–308.

24 McMeekin, T.A., T. Ross and J. Olley. 1992. Application of predictive microbiology to assure the quality and safety of fish and fish products. Int. J. Food Microbiol. 15: 13–32.

25 Murphy, K.P., P.L. Privalov and S.J. Gill. 1990. Common features of protein unfolding and dissolution of hydrophobic compounds. Science 247: 559–561.

26 Neumeyer, K. 1992. Effect of temperature history on predicting the growth responses of *Staphylococcus aureus*. BSc (Hons) Thesis, University of Tasamia.

27 Ohta, F. and T. Hirahara. 1977. Rate of degradation in cool-stored carp muscle. Mem. Fac. Fish. Kagoshima Univ. 26: 97–102.

28 Ratkowsky, D.A. 1990. Handbook of Nonlinear Regression Models. Marcel Dekker, New York.

29 Ratkowsky, D.A. 1992. Principles of nonlinear regression modeling. J. Ind. Microbiol. 12: 195–199.

30 Ratkowsky, D.A., R.K. Lowry, T.A. McMeekin, A.N. Stokes and R.E. Chandler. 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154: 1222–1226.

31 Ratkowsky, D.A., J. Olley, T.A. McMeekin and A. Ball. 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149: 1–5.

32 Ratkowsky, D.A., T. Ross, T.A. McMeekin and J. Olley. 1991. Comparison of Arrhenius-type and Bĕlehrádek-type models for prediction of bacterial growth in foods. J. Appl. Bacteriol. 71: 452–459.

33 Ross, T. 1987. Bĕlehrádek temperature functions and growth of organisms. CSIRO–DSIR Joint Workshop on Seafood Pro-

cessing, Nelson, New Zealand, April 1986. CSIRO Tasmanian Regional Laboratory Occasional Paper No. 18.

34 Ross, T. and T.A. McMeekin. 1991. Predictive microbiology: applications of a square root model. Food Aust. 43: 202–207.

35 Schoolfield, R.M., P.J.H. Sharpe and C.E. Magnuson. 1981. Non-linear regression of biological temperature-dependent rate models based on absolute reaction rate theory. J. Theor. Biol. 88: 719–731.

36 Scott, W.J. 1953. Water relations of *Staphylococcus aureus* at 30 °C. Aust. J. Biol. Sci. 6: 549–564.

37 Smith, M.G. 1985. 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. Hygiene 94: 289–300.

38 Smith, M.G. 1987. Calculation of the expected increases of coliform organisms, *Escherichia coli* and *Salmonella typhimurium*, in raw blended mutton tissue. Epidemiolog. Infect. 99: 323–331.

39 Zwietering, M.H., J.T. de Koos, B.E. Hasenack, J.C. de Wit and K. van't Riet. 1991. Modeling of bacterial growth as a function of temperature. Appl. Environ. Microbiol. 57: 1094–1101.