Summary

Predictive microbiology involves the accumulation of knowledge on microbial growth responses to environmental factors which is summarised as an equation or model. The raw data and model may be stored in a data base from which the information can be retrieved and used to interpret the effect of processing and distribution practices on microbial proliferation. When coupled with information on environmental history during processing and storage, predictive microbiology Provides precision in making decisions on the microbiological safety and quality of foods.

This paper examines the development of predictive microbiology, describes modelling and validation procedures, and identifies and answers commonly perceived problems with the concept. Practical applications in the meat industry are described and the future of predictive microbiology is considered.

Introduction

McMeekin *et al.* (1993), in a monograph on predictive microbiology, introduced the concept by stating that

a detailed knowledge of the growth responses of microorganisms to environmental conditions enables objective evaluation of the effect of Processing, distribution and storage operations on the microbiological safety and quality of foods. If the microbial ecology of a processing Operation or a product during distribution and storage is understood, survival and/or growth of an organism of concern may be predicted on the basis of a mathematical relationship between microbial growth rate and environmental conditions. In turn, this means that the potential exists to estimate food quality and safety by monitoring storage conditions such as temperature or gaseous atmosphere or intrinsic properties of the food such as water activity or pH value.

All embracing statements of this type extolling the virtues and potential of predictive microbiology are commonplace in reviews of the subject. Such reviews are usually written by workers active in the field and no doubt similar sentiments appear in research grant applications (with \$ figures attached) from those who want to continue modelling programs.

However, many food microbiologists remain sceptical that an inherently simple procedure such as measuring a temperature history profile in a processing operation or distribution chain could possibly provide an accurate estimate of shelf life or safety.

Development of predictive microbiology

Impetus for the development of predictive microbiology in the last decade was derived from increased public awareness of food safety issues as a result of major food poisoning outbreaks in several developed countries, including the United Kingdom and the United States. The original use of the term *predictive modelling* may be attributed to Roberts and Jarvis (1983). However, the idea is not new, and the concept was encapsulated by Scott (1937) in the introduction to his classical paper describing the effect of temperature on the growth of meat spoilage organisms:

USE OF PREDICTIVE MICROBIOLOGY IN RELATION TO MEAT AND MEAT PRODUCTS

T.A. McMeekin and T. Ross Department of Agricultural Science, University of Tasmania, Hobart, Tasmania, Australia A knowledge of the rates of growth of certain micro-organisms at different temperatures is essential to studies of the spoilage of chilled beef. Having these data it should be possible to predict the relative influence on spoilage exerted by the various organisms at each storage temperature. Further, it would be feasible to predict the possible extent of the changes in populations which various organisms may undergo during the initial cooling of sides of beef in the meatworks when the meat surfaces are frequently at temperatures very favourable to microbial proliferation.

Scott's (1937) paper is one of a trilogy on the growth of microorganisms on ox muscle published in the period 1936-1938. Scott (1936) discussed the influence of water content of substrate and Scott (1938) examined the effect of carbon dioxide. Combined with investigations on chilled beef (Empey and Scott, 1939; Scott and Vickery, 1939), these studies provided a sound scientific basis to enable the shipment of chilled meat quarters from Australia to the United Kingdom.

The publications also provide a benchmark for current studies in predictive microbiology, viz. a well-defined objective, e.g., the cooling and storage of beef in the meatworks will be analysed with the object of defining the optimum procedure to be adopted when beef is intended for export in the chilled condition (Scott and Vickery, 1939), good experimental design, clearly described methods, collection of sufficient data with rigorous analysis of that data and thoughtful discussion with soundly based conclusions.

Although Scott and his colleagues had developed the concept of predictive microbiology and provided a database, they lacked access to information technology systems to enable precise definition of individual elements of the processing and distribution chain. Nevertheless, they were able to specify combinations of temperature, relative humidity and carbon dioxide content to allow shipments of chilled beef quarters to reach the United Kingdom in an acceptable condition after a voyage of 40-60 days. In today's modelling jargon they had defined the leading edge of a response surface.

The information technology is, of course, now readily available to interpret environmental histories in terms of the extent of microbial growth, provided that:

- the mathematical model describing the response of the organism of concern to changing environmental conditions is valid; and
- the sensor or monitoring device accumulating the environmental information is appropriately placed to provide data that truly reflects the spoilage or public health status of usually heterogeneous products.

These are two of several problems perceived in the application of predictive microbiology which will be considered further in subsequent sections of this paper.

Additional impetus for the adoption of predictive microbiology has come from the perception of many food microbiologists that our discipline is caught in the time warp of agar-based methods (Sharpe, 1980). Further, despite enormous interest in the development of rapid methods, many remain limited in their ability to provide an early warning of spoilage or the presence of dangerous levels of pathogens or their toxins. Current systems based on the production of spoilage metabolites, such as sulphides and amines, characteristically respond at levels approaching incipient spoilage ($\approx 10^7$ cells/cm²), while those based on impedance/conductance changes respond several generations earlier. It remains to be seen how far advances in technology, e.g., biosensors, will lower the limits of microbial detection in foods. The exponential nature of bacterial growth ensures that the *law of diminishing returns* will apply when one generation represents an extremely small decrease in absolute numbers.

For many applications of predictive microbiology, marked advances in concepts or equipment are not required. McMeekin *et al.* (1992) drew parallels between predictive microbiology and Hazard Analysis and Critical Control Point (HACCP) programs. Both are inherently simple systems based on experience with products and processes and supplemented by accumulation and analysis of information at target sites.

Thus, predictive microbiology may be viewed as a data acquisition and retrieval system providing quantitative information for identification, monitoring and regulation of critical control points. The connection between the two approaches is emphasised to indicate a possible route for the widespread acceptance of predictive microbiology as an extension of HACCP programs.

Perceived problems with predictive microbiology

The major problems identified by writers on the subject are:

- varying initial numbers of contaminant microorganisms which will affect the absolute time for spoilage or a public health risk to occur;
- the complexity of food systems in terms of heterogeneity in their composition and the contaminating microbiota, including the possibility of microbial interactions;
- the contribution of non microbial factors to product deterioration;
- construction of a suitable mathematical model to define responses to environmental factors such as temperature, water activity and pH and an inherent distrust of empirical models;
- the effect of the inherent variability of biological systems and the probability of consistent responses, particularly under stressful conditions;
- the effect of changing external conditions such as temperature fluctuations or surface drying; and
- collection of appropriate environmental information such as temperature history profiles.

Specified levels and relative rates

Many of the criticisms of predictive microbiology are based on uncertainty of where the process starts and where it stops, and by thinking in terms of absolute rates rather than relative rates.

The starting point and end point for any application of predictive microbiology may be selected on the basis of accumulated knowledge of the microbiology of a processing operation or a product in storage; e.g., good manufacturing practices in the Australian dairy industry will invariably produce pasteurised, homogenised milk with an initial level of contamination with pseudomonads mL⁻¹. Specifying this level as a starting point is consistent with a shelf life of 8-10 days at 4° C when the pseudomonads will have reached $\approx 10^{7.5}$ mL⁻¹. This is suitable for current storage and distribution practices, and because it is an easily achievable target, the risk to the consumer is minimised. Although 8-10 days at the recommended storage temperature may underestimate the shelf life of many individual packages this is not considered to be a problem. If the product is wholesome by the use-by date, both the consumer and the processor will be satisfied (Chandler and McMeekin, 1989).

Alternatively, an initial level may be specified by obtaining information on a product at a known constant temperature (Gill et al., 1988a), i.e., knowing the assured shelf life at a defined temperature and the number of bacteria required to cause spoilage enables calculation of a notional value for initial numbers. This procedure also takes account of the effect of product composition and packaging on the absolute time to spoilage. In effect, this is a challenge test using the normal spoilage biota and the same rationale could be applied equally well to define the behaviour of pathogenic or indicator organisms occurring naturally or deliberately added for the purpose of the test. Once the extremes have been defined, application of predictive models depends on the relative rate concept to integrate the effect of environmental factors on the behaviour of the organisms of concern (Nixon, 1971; Olley and Ratkowsky, 1973a; 1973b). Appropriate relative rates are derived from mathematical models constructed as described in the following section. The relative rates for psychrotrophic pseudomonads shown in Table 1 are based on the square root model and a theoretical minimum temperature (Tmin) for growth of 265K. There is considerable confidence in selection of this Tmin value which is based on 31 data sets collected from 1961-1993. From flesh foods, the mean value of the data sets was 264.4 (2. 20) and from milk the mean value obtained from 15 data sets was 265.6 (1.24). The overall mean (n = 31) was 265.0 (1.84)(K. Neumeyer and L. Kamperman, personal communication).

Temperature (oC)	Relative rate	
0 2 5 7 10 20 25	0.64 1.00 1.69 2.25 3.24 5.29 10.89	

Table 1. Relative rates of growth for a psychrotrophic pseudomonad (T_{MIN}=265°C) taking 2°C as a reference temperature with relative rate=1.00.

Further, with products such as fish in which other deteriorative processes occur, these have similar T_{min} values (Bremner *et al.*, 1987) and thus show the same relative rate response to temperature. The response to temperature of a particular

organism in meats is unlikely to be altered by microbial interactions as confirmed by the reports of Gill and Newton (1977) for spoilage microbiota and Gill and Newton (1980) for *Salmonella* and spoilers.

The modelling process

An absolute prerequisite for the development of reliable mathematical models is the collection of sufficient good quality data. Because of the quantity and quality of data required, modelling studies are often based on observations made under well defined conditions in laboratory media. Growth rates or lag phase duration may be determined from viable counts or turbidometric or other indirect methods. The values obtained are then used to describe the effect of temperature, water activity or pH on the rate of microbial development using kinetic or probability models.

Readers are referred to McMeekin *et al.* (1993), chapter 2, for a detailed commentary on methods of data collection and analysis.

Taking the situation where temperature is the sole variable, the simple square root model of Ratkowsky *et al.* (1982) provides a starting point.

$$\sqrt{\mathbf{k}} = \mathbf{b}(\mathbf{T} - \mathbf{T}_{min})$$

where k is a growth rate constant, T is the absolute temperature, T_{min} is the notional minimum temperature for growth and b represents the slope of the relationship between \sqrt{k} and temperature.

An extension of the above model for the combined effect of temperature and water activity was proposed by McMeekin et al. (1987)

$$\sqrt{k} = b(T - T_{min}) \sqrt{a_w - a_{wmin}}$$

where a_w is the water activity and a_{wmin} is the theoretical minimum water activity for growth. A model of analogous form was proposed by Adams *et al.* (1991) to describe the combined effect of temperature and pH

$$\sqrt{k} = b(T - T_{min}) \sqrt{pH - pH_{min}}$$

where pH_{min} is the theoretical minimum pH.

A simple model for temperature, water activity and pH was foreshadowed by McMeekin *et al.* (1992)

 $\sqrt{k} = b(T - T_{min}) \sqrt{(a_w - a_{wmin})(pH - pH_{min})}$

and this *multiplicative* theme was developed further by Ross and McMeekin (1993), McMeekin *et al.* (1993) chapter 5, and D. A. Ratkowsky (personal communication). Using data sets kindly supplied by Dr. R.L. Buchanan, U.S.D.A., Philadelphia, Ratkowsky developed a model containing only four parameters to describe the effect of temperature, salt concentration, pH, sodium nitrite level and aerobic or anaerobic conditions on the generation times and lag times of five food-borne pathogenic bacteria. The results obtained by Ratkowsky in this exercise indicated major effects on rates of temperature, water activity and pH but lesser roles for NO₂ concentration and atmosphere.

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The efficacy of models of the type shown above to describe the effects of temperature, water activity and pH has been confirmed by Wijtzes *et al.* (1993) for growth of *Listeria monocytogenes*.

An alternative approach to describe the effect of multiple factors on bacterial growth is to use a low order polynomial (Gibson *et al.*, 1988; Buchanan and Phillips, 1990).

 $\ln Y = a + b_1 S + b_2 T + b_3 P + b_4 S^2 + b_5 T^2 + b_6 P^2 + b_7 ST + b_8 SP + b_9 TP + E$

where Y is a parameter related to the growth rate or lag time derived from data fitted by a Gompertz function (Gibson *et al.*, 1987) and the coefficients *a*, b_1 - b_9 were determined by least squares regression.

The end result of the polynomial approach is a predictive model that contains a large number of parameters, e.g., the model of Gibson *et al.* (1988) for *Salmonella* had 20 constants from three environmental variables, while for *Aeromonas hydrophila* the models of Palumbo *et al.* (1991) had 30 constants from four environmental factors.

When examining individual factors independently, a large number of data points per independent variable can be handled with 10-15 suggested as the minimum number. However, if the effect of several factors is considered simultaneously, as in the polynomial (response surface) approach, specialised experimental designs (e.g., central composite design) are required to reduce the number of experimental points to a manageable level. This may restrict the range of each independent variable examined, or lead to situations where the effects of several constraints on growth result in extremely long generation or lag times, with a marked increase in variability, as discussed below.

Variability in biological responses

Ratkowsky *et al.* (1991) drew attention to the increase in variability of estimates of generation time or lag phase duration as the magnitude of the response increased. This effect was shown clearly by Ratkowsky *et al.* (1991) using the replicated data sets of Smith (1985) for the growth of *Escherichia coli* on meat. Under extremely stressful conditions Cole *et al.* (1990) have shown that the results obtained become increasingly erratic, making predictions of survival times unreliable. Further comment on variability in initiation of growth by *Clostridium botulinum* was made by Graham and Lund (1993) who indicated that with low inocula *no growth may be registered in a situation where growth is indeed possible, but has a low probability*.

The question of variability was addressed in detail by Ratkowsky (1992) who indicated that response variables of interest in predictive microbiology are typically non-normally distributed. Using replicated data sets of Smith (1985) and Neumeyer (1992), the variances were shown to be proportional to the squares or cubes of their means, i.e. the distribution of the data was *gamma* or *inverse Gaussian*, both of which show a strong right hand tail. This has major consequences for variability with increasing response times. For a gamma distribution the mean / variance relationship is given by

where V is the variance, is the mean response time and c is a constant or scale parameter. For an inverse Gaussian distribution the mean/variance relationship is

 $V = c^3$

It is therefore important to recognise the asymmetry of time based variates and it would be misleading to use a symmetric confidence interval as would be the case if estimates of response times are normally distributed.

Using the generation time data of Smith (1985) which followed an inverse Gaussian distribution, Ratkowsky (1992) calculated the variances shown in Table 2.

 Table 2. Redicted generation time and its variance. Data of Smith (1985) for growth of coliforms on meat.

Temperature (oC)	Predicted generation time (hours)	Variance ^z	
10	7.097	5.5492	
15	2.472	0.2345	
20	1.244	0.02989	1.1.1.1.1.1.1
25	0.747	0.006471	
30	0.498	0.001917	
35	0.355	0.000695	
40	0.266	0.000292	

^z V= 0.015524 (generation time)³

The data of Neumeyer (1992) for the growth of *Staphylococcus aureus* strain 3b provides a further example of an inverse Gaussian distribution of time as a response variable (Table 3). Clearly, at temperatures close to the minimum for growth there is a marked increase in variability, and confidence in obtaining correct predictions must decrease.

Under these circumstances a better approach may be to determine the probability of achieving a certain generation time based on the distribution of data (Ratkowsky, 1992). An example of this approach is given in Table 4 which illustrates that only once in 10,000 events would the generation time of *S. aureus* strain 3b exceed 69 minutes, i.e. if this generation time is chosen for predictive modelling purposes the chance of a *fail dangerous* event is one in 10,000.

Similar exercises can be carried out with data that are gamma distributed. Thus, for the citric acid data of Adams *et al.* (1991) at a temperature of 278.5K and pH 5.0, the mean predicted time for a 100-fold increase in numbers was 12.00 days. The probability of a 100-fold increase being achieved in eight days was 10^{-6} ; in nine days, it was 10^{-3} ; and in 10 days, it was 10^{-2} (Ratkowsky, 1992).

Temperature (oC)	Predicted generation time (minutes)	Variance ^z
12.5	887.76	571600
15	388.62	47950
20	138.23	2158
27	56.43	146.8
28	51.03	108.6
30	42.32	61.9
35	28.28	18.5

 Table 3. Predicted generation time and its variance. Data of Neumeye (1992)
 for growth of *Staphylococcus aureus* strain 3b in Brain Heart Infusion broth.

^z V= 0.000817 (generation time)³

Table 4. Values of generation time (minutes) corresponding to the probability of that event occurring. Using data of Neumeyer (1992) for growth of *Staphylococcus aureus* strain 3b in Brain Heart Infusion broth at 12.5°C.

Generation time (minutes)	Probability	
46 55 69 92 136 168 203 657 2356 2951 3782	$\begin{array}{c} 0.000001\\ 0.00001\\ 0.0001\\ 0.001\\ 0.001\\ 0.025\\ 0.05\\ 0.5\\ 0.95\\ 0.975\\ 0.99\end{array}$	

Model validation

When a model has been constructed in laboratory media it requires validation in food systems, preferably under fluctuating temperature regimes that simulate those likely to be encountered in commercial practice.

Some authors have claimed that the temperature history experienced by an organism will have an effect on subsequent growth kinetics, particularly lag phase duration (Walker *et al.*, 1990; Buchanan and Klawitter, 1991; Fu *et al.*, 1992). However, most authors conclude that there is no effect on growth rate (Shaw *et al.*, 1971; Pooni and Mead, 1984; Gill and Harrison, 1985; Gill, 1986; Neumeyer, 1992). McMeekin *et al.* (1993) have suggested that in some cases the observed effect on lag phase may be due to the use of inocula at different stages of population growth, with different time/temperature combinations yielding inocula ranging from mid exponential phase to the equivalent of many generations into

the stationary phase of growth. The latter will obviously display a marked lag phase upon inoculation into fresh media.

Further support suggesting that fluctuating temperatures do not constitute a major problem comes from validation studies not specifically designed to test the effect. Examples of studies in which models have been validated in products with fluctuating temperatures include the unpublished work of Spencer and Baines cited by McMeekin *et al.* (1988) for white fish, Langeveld and Cuperus (1980) for pasteurised milk and Smith (1987) for the growth of *E. coli* on red meat.

Practical applications of predictive microbiology

The construction of suitable models for predictive purposes and their validation are largely laboratory based exercises in which the development of a naturally occurring or deliberately added population is observed under well controlled conditions. There is now ample evidence that it is possible to produce accurate models and to define confidence limits for the predictions derived from those models. The next step is to devise methods by which the accumulated information can be used in practical situations.

One means of achieving this goal is to combine the kinetic information describing microbial growth responses with information on the chemical and physical characteristics of foods and the likely conditions of storage. Expert systems of this type can then be interrogated to estimate the effect of changes in storage conditions or product formulation on microbial development.

An example of this type of system is *Food Micromodel*, the end product of a major research investment by the United Kingdom Ministry for Agriculture, Fisheries and Food. The information pack and promotional video describes *Food Micromodel* as an innovative computer based product which can predict the growth, death and survival of most common food poisoning bacteria in a wide range of foods. Applications suggested for *Food Micromodel* included new product development, ensuring food safety by temperature control, developing hazard analysis programs for food processing and distribution systems and in staff education and training programs.

The Pathogen Modelling Program developed by R. L. Buchanan's group at USDA, Philadelphia, U.S. (Buchanan, 1991) and the decision support system described by Zwietering *et al.* (1991) are other examples of application technology.

However, the practical application of a data base in a truly kinetic sense requires accurate accumulation of data on factors controlling microbial growth during processing and distribution. Several devices used to record temperature history have been discussed by Ross and McMeekin (1991). These range from disposable chemical indicators to electronic integrators or loggers. The former are essentially electronic clocks which keep real time at the recommended temperatures mimicking the bacterial rate response to temperature changes. Integrators provide a continuous display of elapsed time/temperature history as equivalent time at a reference temperature.

Loggers generally do not have this facility but record temperature at intervals. The record may be down loaded as a hard copy and some devices, such as the Delphi system, interpret the temperature history with specific computer software to indicate the extent of growth of an organism of concern.

For some purposes, placement of the sensor to collect appropriate information does not present a problem e.g. with liquid products, or with cartons of beef during cooling, the geometric centre of the unit is selected. However, in other situations heterogeneity in product size and shape and variability in rates of temperature change dictate selection of a site representing the worst case, e.g., in carcass cooling the aitch bone pocket is the slowest cooling surface (Scott and Vickery, 1939; Gill *et al.*, 1991a).

A major application of predictive microbiology with meat and meat products has been to assess the hygienic efficiency of various processing operations. For this purpose the organisms of concern are members of the *Enterobacteriaceae* including indicators, such as coliforms and *Escherichia coli*, and pathogens such as *Salmonella*. These display mesophilic temperature characteristics and the development and validation of temperature dependence models have been described by the authors listed below. Each of these studies used a model based on square root kinetics and ranged from laboratory based observations using artificial media or meat slices to observations carried out in commercial processing operations including offal chilling, carcass cooling and storage and distribution practices.

Gill, 1984	Development of anaerobic model for <i>E. coli</i> growth, validation in offal cooling procedures
Gill and Harrison, 1985	Validation of Gill (1984) model in offal cooling procedures
Smith, 1985	Model of coliforms growing aerobically on meat - implications for codes of practice (laboratory study)
Gill (1986)	As for Gill (1984)
Smith, 1987	Validation of Smith (1985) model in raw blended mutton (laboratory based study)
Mackey and Kerridge, 1988	Models for <i>Salmonella</i> in minced beef (growth rate and lag phase duration). Effect of inoculum size (laboratory study)
Gill <i>et al.</i> , 1988b	Development of computer programs to evaluate process hygiene. Models mentioned for aerobic and anaerobic conditions, lag phase duration and growth rate but no details given
Lowry et al., 1989	Aerobic models for <i>E. coli</i> lag phase duration and growth rate. Validation for meat thawing procedures
Gill and Phillips, 1990	Gill (1984) model validated for offal cooling and Gill <i>et a</i> (1988b) model for carcass cooling. Temperature function integration criteria for carcass cooling

Gill <i>et al.</i> , 1991a	Validation of Gill <i>et al.</i> (1988b) model (details now provided for conventional carcass cooling)
Gill et al., 1991b	Validation as above for spray cooling of carcasses
Reichel <i>et al.</i> , 1991	Validation as above and anaerobic model of Gill (1984) in hot boning processes
Gill and Jones, 1992a	Validation of aerobic <i>E. coli</i> model in cooling of pig carcasses
Gill and Jones, 1992b	Validation of anaerobic model of Gill (1984)

Collectively, the publications illustrated the potential of temperature function integration to predict accurately the behaviour of enteric organisms on meat when temperature was the sole factor controlling growth. This despite a rather unusual *kink* in the square root plot first reported by Gill (1984), reproduced by Gill and Harrison (1985) and perpetuated in many subsequent publications by Gill and colleagues. Fortunately the kink occurred at $\approx 30^{\circ}$ C where rapid rates of growth are transformed into small estimates of time thus having much less effect on the estimated number of generations of *E. coli* than would be the case at low temperatures. McMeekin *et al.*(1993) replotted Gill's data from 7°C to 35°C and concluded that it could be interpreted as a straight line with a correlation Coefficient of 0.99.

The papers listed above, published up to 1988, largely describe model development and laboratory based validation on fairly simple commercial Operations such as offal cooling. Lowry et al. (1989) in examining meat thawing procedures also had to take account of damage caused by freeze/thaw insults, variable rates of warming at different parts of cartons and the development of anaerobic conditions as a result of accumulation of drip from thawed surfaces. This emphasises the need to define exactly those environmental factors limiting microbial growth during a particular processing operation if a correct interpretation of the hygienic adequacy of the process is to be made. The experience of Lowry et al. (1989) and the development of aerobic and anaerobic models for growth rates and lag phase duration by these authors and Gill et al. (1988b) foreshadowed studies of the practical application of temperature function integration on an industrial scale published by Gill and coworkers since 1991. These studies have a standard format with detailed description of product temperature history and integration of the temperature history data with respect to bacterial growth. With one exception each of these studies relied on the veracity of a model appropriate to the process and only Reichel et al. (1991) made a direct comparison of calculated and directly determined growth of E. coli. During the hot boning process under consideration pairs of calculated and observed values were within one generation in 76% of comparisons and calculated estimates were greater than the observed in 60% of comparisons.

For several of the processes examined, (offal chilling, hot boning, spray chilling of carcasses) temperature will undoubtedly be the major constraint on bacterial

growth rate and an appropriate temperature dependence model can be selected to accommodate aerobic or anaerobic conditions or the switch from one atmosphere to the other. However, in the case of conventionally cooled carcasses no account was taken of the effect of surface drying on growth rate which has been cited as an important controlling factor since the work of Scott and Vickery (1939). Gill and Phillips (1990) argue that while evaporation of water from warm carcasses and consequent drying is critically important to microbial stability in traditional butchery practice, in many current processes conditions during cooling are arranged to restrict water loss and that inhibition of bacteria by surface drying is unlikely. Even if this does occur they rationalise that there are invariably areas of carcasses where drying is not sufficient to retard bacterial growth and under these circumstances the *worst case scenario* is the appropriate one upon which to base process assurance decisions.

The argument can only be resolved satisfactorily by experiments to compare calculated and directly determined *E. coli* proliferation in a conventional cooling process, as reported by Reichel *et al.* (1991) for hot boning operations. If drying is shown to have a significant effect a more intractable problem will be to measure accurately the rate of change of surface water activity.

In the interim such arguments should not be allowed to detract from the potential of predictive microbiology to bring objectivity and precision to process hygiene decisions. Ross and McMeekin (1993) described predictive microbiology as more *a philosophical approach than a specific method or technology*, i.e., the prediction does not need to be viewed in absolute terms to provide a useful indication of the hygienic adequacy of a process. This point was appreciated by Gill and Phillips (1990): *Temperature function integration must evaluate the hygienic efficiency of a process; it cannot be used to assess the absolute hygienic status of individual units leaving the process.*

Time-temperature function integration criteria for meat cooling have now been suggested for conventional cooling processes (Gill *et al.*, 1991a), spray chilling processes (Gill *et al.*, 1991b), hot boning processes (Reichel *et al.*, 1991), cooling of pig carcasses (Gill and Jones, 1992a) and cooling of beef offals (Gill and Jones, 1992b).

Fewer studies have been carried out on the application of predictive microbiology to assess the shelf life of meat and meat products (Gill *et al.*, 1988a; Gill and Jones, 1992a). The essential difference is that the organisms of concern are psychrotrophs (pseudomonads under aerobic conditions and enterobacteria under anaerobic conditions) requiring a different temperature dependence model. Otherwise exactly the same considerations apply as described above for evaluation of hygienic adequacy.

Conclusions

Farber (1986) recounted that in 1983 at a symposium of the Society for Applied Bacteriology, a panel of 30 expert food microbiologists using the Delphi technique of intuitive forecasting, predicted that assessment of shelf life by computers, drawing on databases for the growth of spoilage organisms had an 80% probability of being widely used by 1993. At least 25% of the panel, however, did not believe that such an approach would be acceptable even at the beginning of the 21st century (Jarvis, 1983). Time has shown the majority to be correct in that the

concept of predictive microbiology has become reality, at least for some applications.

The authors responsible for this paper, although highly biased and certainly not normally distributed in their enthusiasm for predictive microbiology, now confidently predict increased acceptance of the concept in the food industry. This will come with:

- the refinement of existing models and the development of models for other organisms and different constraints;
- the development of improved or novel strategies for application;
- the development of more sophisticated sensors and increased precision in their placement; and
- the realisation that predictive microbiology is not simply another method specifically designed to improve shelf life or microbiological safety of foods.

Rather it should be viewed as:

- an empowering concept that brings objectivity and precision to decisions pertaining to the microbiological quality and safety of foods;
- a versatile process which can be used to make decisions for multiple purposes including quality assurance, regulation and targeting specific markets; and
- the ultimate rapid method by which any sector of the food industry or a regulatory authority can make an instantaneous decision on the acceptability of material for any future purpose.

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References

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

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

BUCHANAN, R.L. 1991 Using spreadsheet software for predictive microbiology applications. J. Food Safety. 11:123-124.

BUCHANAN, R.L., and KLAWITTER, L.A. 1990. Effect of temperature history on the growth of *Listeria monocytogenes* Scott A at refrigeration temperatures. *Int. J. Food Microbiol.* 12:235-246.

BUCHANAN, R.L., and PHILLIPS, J.G. 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Prot. 53:370-376, 381.

CHANDLER, R.E., and McMEEKIN, T.A. 1989. Temperature function integration as the basis of an accelerated method to predict the shelf life of pasteurized-homogenized milk. *Food Microbiol.* 6:105-111.

COLE, M.B., JONES, M.V., and HOLYOAK, C. 1990. The effect of pH, salt concentration and temperature on the survival and growth of *Listeria* monocytogenes. J. Appl. Bacteriol. 69:63-72.

EMPEY, W.A., and SCOTT, W.J. 1939 Investigations on chilled beef Part 1: Microbial contamination acquired in the meat works. *Counc. Sci. Ind. Res. (Aust.)* Bull. 126.

FARBER, J.M. 1886. Predictive microbiology of food deterioration and safety. In: M.D PIERSON and N.J. STERN (eds). *Foodborne Microorganisms and their Toxins*. Marcel Dekker Inc., p.p. 57-90.

FU, B., TAOUKIS, P.S., and LABUZA, T.P. 1991. Predictive microbiology for monitoring spoilage of dairy products with time-temperature integrators. *J. Food Sci.* 56:1209-1215.

GIBSON, A.M., BRATCHELL, N., and ROBERTS, T.A. 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *J. Appl. Bacteriol.* 62: 479-490.

GIBSON, A.M., BRATCHELL, N., and ROBERTS, T.A. 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. *Int. J. Food Microbiol.* 6:155-178.

GILL, C.O. 1984. Prevention of early spoilage of livers. Proceedings of the 30th European Meeting of Meat Research Workers, Bristol, U.K. p.p. 240-241.

GILL, C.O. 1986. Temperature function integration for hygiene evaluation of food processing procedures. *Food Technol. Aust.* 38:203-204.

GILL, C.O., and HARRISON, J.C.L. 1985. Evaluation of the hygienic efficiency of offal cooling procedures. *Food Microbiol.* 2:63-69.

GILL, C.O., and NEWTON, K.G. 1977. The development of the aerobic spoilage flora on meat stored at chill temperatures. *J. Appl. Bacteriol.* 43:189-195.

GILL, G.O., and D NEWTON, K.G. 1980. Growth of bacteria on meat at room temperature. J. Appl. Bacteriol. 49:315-323.

GILL, C.O., PHILLIPS, D.M., and LOEFFEN, M.P.F. 1988a. A computer program for assessing the remaining storage life of chilled red meats from product temperature histories. In: Refrigeration for Food and People: Proceedings of Meetings of Commissions C2, D1, D2/3, E1 (September 5 - 9, 1988), Institut International du Froid - International Institute of Refrigeration, Paris. p.p.73-77.

GILL, C.O., PHILLIPS, D.M., LOEFFEN, M.P.F., and BISHOP, C. 1988b. A computer program for evaluating the hygienic efficiency of meat processing procedures from product temperature history data. Congress Proceedings: 34th International Congress of Meat Science and Technology, Brisbane, Australia. p.p.531-532.

GILL, C.O., and PHILLIPS, D.M. 1990. Hygienically appropriate time/ temperature parameters for raw meat processing. Congress Proceedings: 36th International Congress of Meat Science and Technology, Havana, Cuba. p.p.458-470.

GILL, C.O., HARRISON, J.C.L., and PHILLIPS, D.M. 1991a. Use of a temperature function integration technique to assess the hygienic adequacy of a beef carcass cooling process. *Food Microbiol.* 8:83-94.

GILL, C.O., JONES, S.D.M., and TONG, A.K.W. 1991b. Application of a temperature function integration technique to assess the hygienic adequacy of a process for spray chilling beef carcasses. *J. Food Prot.* 54:731-736.

GILL, C.O., and JONES, T. 1992a. Assessment of the hygienic efficiencies of two commercial processes for cooling pig carcasses. *Food Microbiol.* 90:335-343.

GILL, C.O., and JONES, S.D.M. 1992b. Evaluation of a commercial process for collection and cooling of beef offals by a temprature function integration technique. *Int. J. Food Microbiol.* 15:131-143.

GRAHAM, A., and LUND, B.M. 1993. The effect of temperature on the growth of non-proteolytic type B *Clostridium botulinum*. *Lett. Appl. Microbiol.* 16:158-160.

JARVIS, B. 1983. Food microbiology into the twenty-first century - a Delphi Forecast. In: T.A. ROBERTS and F.A. SKINNER (eds). *Food Microbiology: Advances and Prospects.* Academic Press, London. p.p.334-367.

LANGEVELD, L.P.M., and CUPERUS, F. 1980. The relation between temperature and growth rate in pasteurized milk of different types of bacteria which are important to the deterioration of that milk. *Neth. Milk Dairy J.* 34:106-125.

LOWRY, P.D., GILL, C.O., and PHAM, Q.T. 1989. A quantitative method of determining the hygienic efficiency of meat thawing processes. *Food Aust.* 41:1080-1082.

MacKEY, B.A., and KERRIDGE, A.L. 1988. The effect of incubation temperature and inoculum size on growth of salmonellae in minced beef. *Int. J. Food Microbiol.* 6:57-66.

McMEEKIN, T.A., CHANDLER, R.E., DOE, P.E., GARLAND, C.D., OLLEY, J., PUTRO, S., and RATKOWSKY, D.A. 1987. Model for the combined effect of temperature and water activity on the growth rate of *Staphylococcus xylosus*. *J. Appl. Bacteriol.* 62:543-550.

McMEEKIN, T.A., OLLEY, J., and RATKOWSKY, D.A. 1988. Temperature effects on bacterial growth rates. In: M.J. BAZIN AND J.I. PROSSER (eds). *Physiological Models in Microbiology*. CRC Press Inc., Boca Raton, Florida. p.p.75-89.

McMEEKIN, T.A., OLLEY, J., ROSS, T., and RATKOWSKY, D.A. 1993. *Predictive Microbiology: Theory and Application*. Research Studies Press, Taunton. 340p.p.

McMEEKIN, T.A., ROSS, T., and OLLEY, J. 1992. Application of predictive microbiology to assure the quality and safety of fish and fish products. *Int. J. Food Microbiol.* 15:13-32.

NEUMEYER, K. 1992. Effect of temperture history on predicting the growth response of *Staphylococcus aureus*. BSc(Hons) Thesis, University of Tasmania.

NIXON, P.A. 1971. Temperature integration as a means of assessing storage conditions. In: *Report on Quality in Fish Products*. Seminar No. 3, Fishing Industry Board, Wellington, New Zealand. p.p. 34-44.

OLLEY, J., and RATKOWSKY, D.A. 1973a. Temperature function integration and its importance in the storage and distribution of flesh foods above the freezing point. *Food Technol. Aust.* 25:66-73.

OLLEY, J., and RATKOWSKY, D.A. 1973b. The role of temperature function integration in monitoring of fish spoilage. *Food Technol.* (*N.Z.*) 8:13,15,17.

PALUMBO, S.A., WILLIAMS, A.C., BUCHANAN, R.L., and PHILLIPS, J.G. 1991. Model for the aerobic growth of *Aeromonas hydrophila* K144. *J. Food Prot.* 54:429-435.

POONI, G.S., and MEAD, G.C. 1984. Prospective use of temperature function integration for predicting the shelf-life of non-frozen poultry-meat products. *Food Microbiol.* 1:67-68.

RATKOWSKY, D.A. 1992. *Predicting response times in predictive food microbiology*. Occasional Paper No.1992/1. Department of Primary Industry, Fisheries and Energy, Tasmania, Research and Development Unit, Biometrics Section. 38p.p.

RATKOWSKY, D.A., OLLEY, J., McMEEKIN, T.A., and BALL, A. 1982. Relationship between temperature and growth rate of bacterial cultures. *J. Bacteriol.* 149:1-5. RATKOWSKY, D.A., ROSS, T., McMEEKIN, T.A., and OLLEY, J. 1991. Comparison of Arrhenius-type and models for prediction of bacterial growth in foods. *J. Appl. Bacteriol.* 71:452-459.

REICHEL, M.P., PHILLIPS, D.M., JONES, R., and GILL, C.O. 1991. Assessment of the hygienic adequacy of a commercial hot boning process for beef by a temperature function integration technique. *Int. J. Food Microbiol.* 14:27-42.

ROBERTS, T.A. and JARVIS, B. 1983. Predictive modelling of food safety with particular reference to *Clostridium botulinum* in model cured meat systems. In: T.A ROBERTS and F.A. SKINNER (eds). *Food Microbiology: Advances and Prospects.* Academic Press, London, pp. 85-95.

ROSS, T., and McMEEKIN, T.A. 1991. Predictive microbiology: Applications of a square root model. *Food Aust.* 43: 202-207.

ROSS, T., and McMEEKIN, T.A. 1993. *Predictive Microbiology: A Review of Recent Advances in Microbiology*. Australian Society for Microbiology, National Examinations Board, Melbourne. (At press).

SCOTT, W.J. 1936. The growth of micro-organisms on ox muscle. I. The influence of water content of substrate on rate of growth at -1°C. J. Coun. Sci. Ind. Res (Aust.). 9:177-190.

SCOTT, W.J. 1937. The growth of micro-organisms on ox muscle. II. The influence of temperature. J. Coun. Sci. Ind. Res. (Aust.). 10:338-350.

SCOTT, W.J. 1938. The growth of microorganisms on ox muscle. III. The influence of 10% CO₂ on rates of growth at -1°C. J. Coun. Sci. Ind. Res. (Aust.). 11:266-277.

SCOTT, W.J., and VICKERY, J.R. 1939. Investigation on chilled beef. Part II. Cooling and storage in the meat works. *Coun. Sci. Ind. Res. (Aust.)*. Bull. 129.

SHARPE, A.N. 1980. Food Microbiology: A Framework for the Future. C.C. Thomas Springfield, Illinois. 224 p.p.

SHAW, M.K., MARR, A.G., and INGRAHAM, J.L. 1971. Determination of the minimal temperature for growth of *Escherichia coli. J. Bacteriol.* 105: 683-684.

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. Hyg. (*Cambridge*). 94:289-300.

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

WALKER, S.J., ARCHER, P., and BANKS, J.G. 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. Appl. Bacteriol.* 68:157-162.

WIJTZES, T., McCLURE, P.J., ZWIETERING, M.H., and ROBERTS, T.A. 1993. Modelling bacterial growth of *Listeria monocytogenes* as a function of water activity, pH and temperature. *Int. J. Food. Microbiol.* 18:139-149.

ZWIETERING, M.H., DE KOOS, J.T., HASENACK, B.E., de WIT, J.C., and van't RIET, K. 1991. Modelling of bacterial growth as a function of temperature. *Appl. Environ. Microbiol.* 57:1094-1101.

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