Review

Predictive food microbiology for the meat industry: a review

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Abstract

Predictive food microbiology (PFM) is an emerging multidisciplinary area of food microbiology. It encompasses such disciplines as mathematics, microbiology, engineering and chemistry to develop and apply mathematical models to predict the responses of microorganisms to specified environmental variables. This paper provides a critical review on the development of mathematical modelling with emphasis on modelling techniques, descriptions, classifications and their recent advances. It is concluded that the role and accuracy of predictive food microbiology will increase as understanding of the complex interactions between microorganisms and food becomes clearer. However the reliance of food microbiology on laboratory techniques and skilled personnel to determine process and food safety is still necessary. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Traditionally the microbiological safety of foods has been established via challenge tests. These tests simulated the effects of environmental conditions on food, in terms of growth and proliferation of spoilage and pathogenic microorganisms. Challenge tests can provide data useful in determining the safety and shelf-life of food under set conditions. However, challenge tests have been criticised as an expensive, labour intensive, time consuming and non-cumulative research tool. More recently challenge tests have been considered as only giving modest assurance on product safety in the food chain (Notermans and Veld, 1994; Baranyi and Roberts, 1995; Roberts, 1997).

The increasing number and severity of food-poisoning outbreaks world-wide has increased public awareness about the safety of meats (Maurice, 1994). Public awareness has been stimulated further by the recent scares of BSE in the UK and *Escherichia coli* 0157:H7 in the US and Scotland. Consumer pressure for greater varieties of minimally processed non-shelf stable and fresh foods has generated a need to quickly and accurately guarantee food safety. Roberts and Jarvis (1983) challenged

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conventional microbiological methods of assessing food safety, as an expensive and largely negative science. An alternative methodology was proposed in which a greater understanding of microbial physiology and the responses of microorganisms to critical controlling factors such as temperature would be required. In this methodology specific microorganisms grow in laboratory media, subjected to various environmental conditions with their responses recorded. Cumulative databases are built up, and procedures to interpolate and interact the database in mathematical models are then developed. This is made under the premise that these responses to environmental conditions are consistent and reproducible (Ross and McMeekin, 1994). However, it is important to note that modelling normally will not show unanticipated microbial responses but it can show effects of multiple variables not specifically tested for (Cole, 1991; Hedges, 1991). Ultimately challenge tests could be fully or in part replaced by a methodology which is embraced by the description predictive food microbiology (PFM) (Whiting, 1997).

PFM is a promising and rapidly developing area of food microbiology, which has gained significant scientific attention in recent years. It encompasses such areas as mathematics, engineering, chemistry and microbiology to give microbial behavioural predictions in specific foods under defined conditions (Zwietering et al., 1990, 1993; Whiting and Buchanan, 1994; Peleg, 1997; Schaffner and Labuza, 1997). In the last 15 years, with the advent of personal computers its real potential and application as an assistive tool to the food industry has been realised. However, despite the progress made by PFM it remains primarily a research rather than an industrial tool. The reasons for this vary but a major problem is that models are often validated against growth of specific organisms in laboratory media under specific conditions (McMeekin et al., 1987). Models can then have difficulties in making accurate predictions from actual food products. As Gill (1982) indicated, variation is inevitable with complex foods such as meat. Validation of models with data from specific meat products is, therefore, recommended (Houtsma et al., 1996). In addition, the judgement of a trained experienced microbiologist will not become redundant by predictive microbiology (Giannuzzi et al., 1998). This paper provides a

critical review on the recent developments in PFM with emphasis on its application and integration into the meat industry

2. Microbiology of meats

Meat is a highly perishable food product which, unless correctly stored, processed, packaged and distributed, spoils quickly and becomes hazardous due to microbial growth. Potential for microbial contamination is influenced by the condition of animals prior to slaughter, abattoir practices, extent of handling and subsequent storage conditions (Jackson et al., 1997). All raw meat can have some level of microbial contamination present and cannot be expected to be otherwise without further processing. However, only if spoilage microorganisms such as *Brochothrix thermosphacta*, *Pseudomonas* spp., and lactic acid bacteria are allowed to grow to high numbers the meat becomes spoiled and unfit for human consumption (Davies, 1992). Depending on the species and whether they are present, pathogens such as *Listeria monocytogenes*, *Salmonella* spp., and *E. coli* 0157:H7 can grow and cause illness by the ingestion of the bacterial cells themselves or from toxins that they produce. The presence of pathogens in the food supply in low numbers is undesirable and is considered a major cause of gastrointestinal disease world-wide (Buchanan and Whiting, 1986).

Within the meat industry, assurance of meat safety and quality are of paramount importance. As the industry develops new technologies to produce higher quality and diverse meat products for increasingly competitive markets, systems must be designed to allow safeguards to be implemented into processing procedures. Traditional approaches to meat safety and quality have relied heavily on regulatory inspection and sampling regimes. However, these systems cannot guarantee total consumer protection unless 100% inspection and sampling are employed. In the meat industry, this level of inspection is impractical for various economic and logistic reasons (Armitage, 1997).

The combination and interaction of the intrinsic and extrinsic factors determines the microbiology of meat. Table 1 lists most of these factors. Among them some factors are especially influential to micro-

Table 1
Some intrinsic and extrinsic factors affecting microbial growth

Intrinsic factors	Extrinsic factors
pH, acidity, acidulant identity, % buffering power	Temperature
Water activity and content, humectant identity	Relative humidity
Redox potential	Light intensity and wavelength
Presence of antimicrobials	Atmospheric gas composition and ratio
Identity and distribution of natural microbial flora	Packaging characteristics and interactions
Presence of physical structures	Processing characteristics and interactions
Presence of biological structures	Storage, distribution and display considerations
Availability of nutrients	
Colloidal form	
Substrate surface to volume ratio	

bial growth in meats. Table 2 indicates how these factors can influence the production of cured cooked meats. The intrinsic nature of most raw meats with high water activities (> 0.98), moderate pH (5.5–6.5) and readily available sources of energy, carbon and other nutrients, makes them ideal for most microbial growth (Varnam and Sutherland, 1985). The most important factor influencing microbial

Table 2
Some intrinsic and extrinsic factors affecting microbial growth in perishable cooked, cured meats

Factors	Affected by
Intrinsic	
pH	Type and level of carbohydrate addition, such as sugar allowing lactic floral growth, use of acidulants or phosphates
Water activity	Salt from brine solution and presence of sugars can alter water activity. Initial and final moisture contents of meat
Antimicrobials	Residual nitrite in final product affected by product pH, temperature and times of processing and storage, iron level of product, growth of nitrite depleting microbes. Level of salt added in cure Use of ascorbate, phosphates or other additives, such as smoke flavours
Initial microbial flora	Type of meat used, slaughtering techniques employed, handling, staff hygiene training, good manufacturing practices
Extrinsic	
Processing parameters	Method of cooking and cooling with times and temperatures achieved
Storage, distribution and display conditions	Time, temperature, relative humidity, packaging and atmosphere histories
Microbial flora	Type and level of natural microbial flora remaining in the product after processing or due to post-process contamination

growth is temperature and it is the primary extrinsic controlling factor. This is evident in most Hazard Analysis Critical Control Point (HACCP) systems for meat products where temperature is a Critical Control Point (CCP) (ICMSF, 1988). For example with the preparation of cooked cold meat, cooking is immediately followed by refrigeration. Governments often provide guidelines or regulations to govern temperature and time necessary in such operations (Anonymous, 1989, 1991; Gaze et al., 1998). The rapid cooling of carcasses, subsequent refrigerated storage and adequate cooking procedures are essential in reducing bacterial growth, increasing shelf-life potential, quality and safety of meats (Hayes, 1985; Smulders and Eikelenboom, 1987).

The consequences of current and future technological operations in meat production are alterations in chemical, physical and microbiological properties of the meat. These alterations can select for a dominant microbial population or species in a meat product. Increasing consumer trends and catering interest towards minimally processed and additive free product has increased potential risks associated with pathogenic growth. As there is a great diversity of microorganisms found on raw meats, processing can reduce microorganisms to a commonality of well-adapted and safe organisms. However, a change or failure in the control system can allow the emergence or re-emergence of a pathogen (Miller et al., 1998). How PFM can deal with such an event remains to be seen.

The large variety of meat products with various methods of processing, packaging, storage and distribution means that there is no single microbiology for meats. However, microbial responses to significant intrinsic and extrinsic factors, such as temperature and curing ingredients in a fresh ham, for example, can be summarised as a simple predictive equation and be used to provide objective assessments of the risks of food borne microbial infection, intoxication or spoilage in specific circumstances (Ross and McMeekin, 1995).

Predictive modelling that integrates microbial behaviour in meat products has now begun to find favour with meat process engineers (Van Gerwen, 1999, personal correspondence). Multifactorial modelling that integrates microbial behaviour could result in an ability to identify the components of most and least significance to the overall fate of a chosen microorganism. For example, one could test the contribution of interacting variables such as pH, acid type, water activity and temperature on the growth of a starter culture in a fermented meat product and identify the component that least or most affects growth. This can facilitate optimisation of the process procedures since it allows engineering of the parameters to give most benefit in terms of cost, quality and convenience (Zwietering and Hasting, 1997b). Leistner (1985) introduced the concept of hurdle technology for meat products. The approach allowed the safety of meat to be guaranteed while maintaining a consumer acceptable product (Leistner, 1985, 1986). However, some intrinsic factors such as ingredient composition will be outside the control of a meat producer because of legislative requirements or customer expectations. In these cases, use will have to be made of extrinsic controls such as packaging atmosphere and storage temperature (Gibbs and Williams, 1990).

Despite the recent improvements in meat production, cases of food poisoning world-wide continue to rise in most countries (Kuhn, 1999). New procedures such as modified atmosphere packaging still require careful temperature control throughout the whole distribution chain. Products such as fresh meats often have extended shelf-life due to modified atmosphere packaging. The use of this technology to retard the growth of rapidly growing spoilage organisms needs to consider the danger posed by slow growing psychrotrophic organisms over longer shelf-life. The need for greater improvements in production of meat products requires microbiological techniques to advance along with other disciplines. Traditional microbiological methods are normally only relevant to the particular conditions under which they were tested and are, therefore, of limited predictive value (Baird Parker and Kilsby, 1987). PFM using mathematical models could overcome these limitations and become a very effective part of meat production and the prevention of food poisoning outbreaks. Unnecessary spoilage of product could be controlled and reduced with its application.

3. Classification of models

A predictive food microbiological model is a mathematical expression that describes the growth,

survival, inactivation or biochemical process of a foodborne microorganism. The area of PFM has several different model classification schemes. However, an absolute classification scheme has yet to be decided. Uniform use of terminology and classification of models into groups which have specific functions makes predictive microbiology a more exact discipline and more user-friendly (Baranyi and Roberts, 1992). However, only the classification system proposed by Whiting and Buchanan (1993) can group most model types together into primary, secondary and tertiary based models (Table 3). Different classification schemes have their advantages and disadvantages.

3.1. Kinetic and probability models

Within predictive modelling, the use of particular mathematical functions is a means of classifying models. Most importantly a model is kinetically or probability based. The choice of approach and the specific application within an approach is largely determined by the type of microorganisms expected to be encountered and the number of variables. Kinetic models predict the extent and rate of growth of a microorganism (Buchanan, 1993a).

Kinetic models can differ in their approach. One approach is to model the growth rate of an organism and use it to make predictions based on the exponential growth of that microbial population. Another approach is to fit a sigmoid function or curve to a microbial population growth data, and then model the effects of various environmental factors such as temperature on this function. Evaluation of this fitted sigmoid curve may allow researchers to make predictions about the studied microorganisms in a particular food system. In both approaches models are constructed by carefully evaluating data collected on increases in microbial biomass and numbers, under a studied criterion of intrinsic and extrinsic parameters such as temperature, pH or a_w. This allows researchers to make predictions about the studied microorganisms lag time, generation time or exponential growth rate (Broughall et al., 1983; Zwietering et al., 1991; Dickson et al., 1992; Van Impe et al., 1995).

Kinetic models attempt to explain the time taken for a specified growth response, in terms of environmental variables such as temperature, pH or a_w (Van Boekel, 1996). Other variables can also be included such as gaseous atmosphere, redox potential (Eh), biological structure, relative humidity, nutrient content and antimicrobial properties. Kinetic models are useful in that they can be used to predict changes

Table 3
Classification of some models used[a]

Primary models	Secondary models	Tertiary models
Gompertz function[1]	Belehradek model *(square-root model)*[10]	USDA Pathogen[18] Modelling Program
Modified Gompertz[2]	Ratkowsky model *(square-root model)*[11]	Food MicroModel[19]
Logistic model[3]	Arrhenius model[12]	Pseudomonas Predictor[20]
Baranyi model[4]	Modified Arrhenius models *(Davey or Schoolfield)*[13]	Expert Systems[21]
First-order monod model[5]	Probability models[14]	
Modified monod model[6]		
D values of thermal inactivation[7]	*Z* values[15]	
Growth decline model of	Polynomial or response[16]	
Whiting and Cygnarowicz[8]	Surface models	
Three-phase linear model[9]	Williams–Landel Ferry model[17]	

[a] 1, Jeffries and Brian (1984), Gibson et al. (1987); 2, Zwietering et al. (1990); 3, Jason (1983), Einarsson and Ericksson (1986); 4, Baranyi et al. (1993a); 5, Monod (1949); 6, Houtsma et al. (1996); 7, Brennan et al. (1990); 8, Whiting and Cygnarowicz Provost (1992); 9, Buchanan et al. (1997), Garthright (1997); 10, Belehradek (1930); 11, Ratkowsky et al. (1982); 12, Arrhenius (1889), Labuza and Riboh (1982); 13, Davey (1989a, 1993a), Schoolfield et al. (1981); 14, Hauschild (1982); 15, Brennan et al. (1990); 16, Draper (1988), Gibson et al. (1988); 17, Williams et al. (1955), Schaffner (1995); 18, Buchanan (1991); 19, McClure et al. (1994b); 20, Neumeyer (1994), Neumeyer et al. (1997a); and 21, Zwietering et al. (1992), Voyer and McKellar (1993).

in microbial numbers with time, even if a controlling variable, which can affect growth, is changing. However kinetic models can be difficult to construct as they require many data from microbial counting to be accumulated (Gibson et al., 1988; McClure et al., 1994a; Baranyi et al., 1995). Good reviews of kinetic modelling are available (Baranyi and Roberts, 1994, 1995; Skinner and Larkin, 1994; Kovarova-Kovar and Egli, 1998).

The use of probability in PFM takes advantage of the likelihood that a particular event will occur under prescribed conditions, as that ability to predict likely occurrences in food systems has obvious advantages. Probability-based models have tended to be used to model spore forming bacteria such as probability of *Clostridium botulinum* survival in canned corned beef (Buchanan, 1993a). The basis for probability modelling is the relationship between the growth of microbial cells and the physico-chemical properties of the environment (Ross and McMeekin, 1995). Probability of growth can help a manufacturer make informed decisions about a product formulation, processing, packaging and storage (Roberts, 1997). Probability models are appropriate in instances where toxin production in a food is of concern, but they provide little information regarding growth rate (Gibson and Hocking, 1997). However, a problem with probability is that probability changes with time, so probability models are in fact a combination of both probability and kinetics and that can make them confusing.

Since the early 1920s, the canned food industry has made use of thermal destruction models to assess the risk of *Cl. botulinum* toxigenesis (Brennan et al., 1990). Standard thermal processing of low acid canned food requires a first-order kinetics heat inactivation model with a 12-decimal reduction (12D) of *Cl. botulinum* spores or 121°C/15 psi for 15 min (Baker, 1993). However, many meat products would be completely inedible even after 1 min at 121°C (Baker and Genigeorgis, 1993). Products such as canned luncheon meats normally receive only a mild heat treatment but remain shelf stable due to incorporation of nitrites, salt and other ingredients to inhibit *Cl. botulinum* spore growth or toxin formation (Genigeorgis, 1986). The use of D values as a response variable merely describes the effects of thermal processing. Pioneering work on probability models has been carried out in both the UK and US. (Genigeorgis et al., 1971a,b; Genigeor-

gis, 1981; Roberts et al., 1981a–c, 1982; Roberts and Gibson, 1986; Roberts, 1989; Baker and Genigeorgis, 1990; Genigeorgis et al., 1991; Meng and Genigeorgis, 1993).

It has been argued that the concept of D value is not sufficient and the ratio of spore recovery after incubation should be considered in calculations used in thermal processing of food. This is particularly relevant to low acid meat products, which receive only minimal heat treatment. Mafart and Leguerinel (1997) presented a model describing the recovery of spores as a function of both heat treatment intensity and environmental conditions and an application may be found with the model in similar types of meat products.

Ross and McMeekin (1994) suggested that the traditional division of PFM into probability and kinetic models was artificial. They argued that the two types of model represented opposite ends of a spectrum of modelling requirements, with research at both ends eventually coming together. Many models to date have concentrated on using kinetic principles rather than probability to model the effects of intrinsic and extrinsic variables particularly temperature on microbial growth rather than survival or death (McMeekin et al., 1997). In certain situations at growth extremes, no growth can often be observed but a small probability of growth can exist (Graham and Lund, 1993). Ross and McMeekin (1995) illustrated some problems in PFM, which are of practical implication for application of kinetic models. Of particular concern to meat products for example would be difficulties in obtaining accurate data on environmental conditions such as temperature at all stages of production, particularly chilling of carcasses (Gill et al., 1991a,b). Variability of microbial response times such as lag phase can also present problems (McMeekin et al., 1989). Therefore, the implication of kinetic model application is that microbial variability increases with increasing response times, thus increasing the confidence limits associated with predictions (McMeekin et al., 1997).

However Ratkowsky et al. (1996) indicated that if the probability distribution of the response time is known, it is possible to determine the probability that an organism will grow more quickly than a predicted response. Therefore, kinetic models can describe consistent microbial growth responses, but under certain conditions, a probability model may be necessary. At near growth limiting conditions a

kinetic modeller has to consider the probability of a predicted growth rate, or no growth (Ross and McMeekin, 1995).

Ratkowsky and Ross (1995) hypothesised that a kinetic model could be used to generate a probability model to describe the growth/no growth area. A logistic regression model to define the probability of growth for several conditions including pH, temperature, salt and sodium nitrite was proposed. A Belehradek kinetic model was used with data for the observable growth of *Shigella flexneri* over a 24-h period. From the model, the boundary between growth and no growth at a particular level of probability could be estimated. However, the model was limited by the use of data, which was not based on growth/no growth conditions.

Presser et al. (1998), supported the above hypothesis (Ratkowsky and Ross, 1995). However, unlike the former actual growth/no growth data was used to differentiate the ability of *E. coli* M23 to grow or not grow under specified environmental conditions. The joining of kinetic and probability approaches can be seen as an integration of kinetic and probability models to PFM and a unification of PFM and the hurdle concept (Ratkowsky and Ross, 1995). However, the reasons for the apparent link between kinetic and probability models are still unclear (Presser et al., 1998). Ratkowsky and Ross (1995) concluded that the suggested link between the models might have been an artefact resulting from using time-limited kinetic data to test a probability model. Models describing the growth/no growth area could be very beneficial to the meat industry.

Products could be formulated having minimum requirements for preservation while satisfying consumer preferences (Presser et al., 1998). Houtsma et al. (1996) described the combined effects of temperature, pH and sodium lactate on the growth of *Listeria innocua* in bologna sausage, in the area that allowed for growth up to the minimum inhibitory concentration of sodium lactate. In this way, it was possible to discriminate between growth and no growth and estimate time in which growth might occur under specific conditions (Houtsma et al., 1993, 1994).

3.2. Empirical and mechanistic models

Empirical models such as the Gompertz function (Jeffries and Brian, 1984), are concerned with practical consequence and simply describe data under experimental conditions in the form of a convenient mathematical relationship (Gibson et al., 1987). Polynomial equations are the common empirical models. These models are easy to use, straightforward and no knowledge of a particular process is required (Whiting, 1995). However, polynomials often have no theoretical foundation and are nonlinear, which are valid only for the range of variables of the underlying data and have numerous parameters without biological meaning. Therefore, polynomials models do not contribute any knowledge to mechanisms underlying a process. (Draper, 1988; Delignette-Muller et al., 1995).

Understanding underlying mechanisms governing cellular metabolism, which produces data, may in time allow the construction of mechanistic models. Models such as this will represent that mechanism more accurately and will serve as a vehicle for generating predictions from hypotheses (Bazin and Prosser, 1992). Interpretation of the modelled response in terms of known phenomena and processes may then be possible (Krist et al., 1998). Baranyi and Roberts (1994) indicated that mechanistically derived models would be easier to develop further, as the quantity and quality of information from the analysed system increases. However completely mechanistic models, which incorporate all intrinsic and extrinsic variables, that affect growth, have not been developed (Labuza and Fu, 1993; Ross et al., 1993).

Most researchers agree that mechanistic models are inherently superior to empirical models for the above reasons (Van Impe et al., 1992, 1993; Zwietering et al., 1993). Currently available models are either empirical or semi-mechanistic (Table 3). If these models are to be continued to be used, it is advisable that models are developed which reflect current knowledge of microbial dynamics and are constructed to provide qualitative data (Baranyi et al., 1996a). However, with the use of more precise microbiological techniques, the demand for empirical growth models may decrease (Whiting, 1992).

3.3. Primary, secondary and tertiary models

Davey (1992) called for a terminology for models to give express meaning to model description and development. Whiting and Buchanan (1993) in response and understanding the necessity to avoid

confusion proposed a new classification system for PFM according to specific criteria under three levels of primary, secondary and tertiary models.

Primary models describe the change of the bacterial number with time under particular environmental and cultural conditions. Response can be measured directly by total viable count (TVC), toxin formation, substrate level or metabolic products or indirectly by absorbance, optical density or impedance. If a bacterial growth curve is monitored by recording how its TVC changes with time the data collected can be plotted using a primary model. This can then generate information about the microorganism such as generation time, lag phase duration, exponential growth rate and maximum population density (Whiting, 1995; Whiting and Buchanan, 1993, 1994).

Secondary models describe the response of one or more parameters of a primary model (lag phase duration) changing to one or more changes in cultural or environmental conditions (pH, a_w, Eh, temperature). For example, if the effects of temperature on the growth of *Salmonella typhimurium* on beef between 15 and 40°C were being investigated, the organism would be grown at a number of temperatures in this range. From each temperature, a generation time can be calculated by using a primary model. These data is then collated using a secondary model, so that effect of temperature is described by a mathematical equation. This allows the end user to determine what generation time will be observed at a temperature T (Dickson et al., 1992; Whiting and Buchanan, 1993, 1994, 1997; Whiting, 1995; Gibson, 1998, personal correspondence).

Tertiary models basically take modelling to its final form. They are applications of one or more primary and secondary models, incorporated into a user-friendly computer software package. These models are incorporated into various function integrators such as temperature, a_w or pH. A time/function integrator history of a product can then be used in conjunction with the secondary model to determine the extent and rate of growth of the organism. Microbial responses to variable conditions and the comparison and contrasting of these effects on several species of microorganism can also be undertaken, using a pertinent database. End users of these systems need not be aware of modelling techniques or the underlying primary and secondary models used Tertiary models make predictive micro-

biology an easily accessible and powerful tool to all areas of food industry and research (Buchanan, 1993b; Whiting and Buchanan, 1993, 1994; Whiting, 1995).

There are several microbial modelling software packages currently available. In the US, the Department of Agriculture (USDA) Food Safety Research Unit has developed the Pathogen Modeling Program. This software uses multivariant models based on the use of the Gompertz function in combination with response surface analysis. It was developed using extensive experimental data on the behaviour of microorganisms in liquid media (Buchanan, 1991, 1993b; Gibson, 1997, personal correspondence). The new version of the software issued in January 1998 contains a growth model for *Clostridium perfringens*, thermal inactivation model for *Cl. botulinum* and gamma irradiation models for *Salmonella*, *E. coli* O157:H7 and normal flora in meats as new features (Whiting and Buchanan, 1997).

The United Kingdom's Ministry of Agriculture, Fisheries and Food (MAFF) has created the Food MicroModel software package which was launched in 1992 (McClure et al., 1994b). This modelling software uses the results of predictive microbiological research in the context of an expert system (Adair and Briggs, 1993; Jones, 1993; Voyer and McKellar, 1993). The Food MicroModel provides a range of predictive models for at least 12 implicated foodborne pathogens.

Although wide ranges of variables affect microbial growth in foods, models are based primarily on the major determinants of microbial growth temperature, pH and water activity, with data for model generation collected using laboratory media (McClure et al., 1994b; Anonymous, 1995). Validation of models for use in food was achieved by comparing the predicted behaviour for each organism against data found in literature for that organism in foods. Where literature was insufficient, experiments were conducted specifically for this purpose of validation (McClure et al., 1994b). Validation of a model in the prediction of growth in a food is based on generation time and time for a specific change in numbers. Lag time models are generally not validated as experience has shown that estimation of lag is generally less repeatable than generation time (McClure et al., 1994b).

Recently a new software package has been laun-

ched, which unlike the others models spoilage pseudomonads rather than pathogens. The Pseudomonas Predictor was developed by a group of scientists at the University of Tasmania in Australia. The software package is spread sheet based and can be used to predict the growth of *Pseudomonas* species. It is also capable of quantifying effects of storage temperature on dairy products, and it can predict how quickly pseudomonads will grow. In addition the software is able to correlate shelf-life of a product to its temperature history. However the Pseudomonas Predictor requires the user to have some computer literacy, which can offset the flexibility of the package (Neumeyer, 1994; Neumeyer et al., 1997a).

With all these software packages there can be no guarantee that predicted values will match those seen in any specific food system. Researchers have pointed out the inadequacies in current information bases and the need for further research and development of predictive modelling software. However research has shown that predictions made with various software packages can agree reasonably well with results from literature or practical experimentation. McClure et al. (1997) also found that predictions from the Food MicroModel compared favourably with results taken on the effects on growth of *L. monocytogenes* by sodium chloride, pH, storage temperature, and sodium nitrate. Comparable results have been seen in other research work, in the comparison of challenge test results and predictions made using predictive microbiological software (Niyoyankana et al., 1995). It was indicated that the predictions made by the Pathogen Modelling Program and the Food MicroModel gave too high growth rates and too long lag phases in predicting growth of *L. monocytogenes* in seafood. The effect of parameters other than those used to obtain predictions was postulated as being the source of the errors in the predictions. This illustrates that a predictive models usefulness and accuracy should be product related (Dalgaard and Vigel Jorgensen, 1998).

Some researchers have proposed the use of computational neural networks for predictive microbiology, but little work relevant to their use in predictive microbiology has been seen. This could be due to the only recent proposal of its use and the lack of appreciable research works in the field (Najjar et al., 1997). In comparing neural networks with predictions made by regression equations for ex-

perimental data pertaining to anaerobic growth of *S. flexneri*, neural networks provided better agreement with the data (Hajmeer et al., 1997). Geeraerd et al. (1998) studied the combined effects of temperature, pH and percent sodium chloride on bacterial growth in chilled foods and indicated that artificial neural networks were a low complex non-linear modelling technique that could accurately describe experimental data in the field of secondary models in predictive microbiology. This modelling technique could make it possible to describe more accurately the interaction of intrinsic and extrinsic variables in chilled meats when compared to more familiar predictive microbiological models. The model was more accurate than the polynomial relationship found in literature. This was explained by the flexible basis functions used in artificial neural network modelling compared to the fixed basis functions of polynomial or any other linear modelling approach.

Apparently, there are many more modelling forms available for describing the same phenomenon. No system of classification is inherently superior to all others. The scheme proposed by Whiting and Buchanan (1993) can simplify the matters. Table 3 summaries the classification of some models used according to this scheme.

4. Description of main models

4.1. Modelling growth curves and determination of growth kinetic parameters (primary models)

Over the last 10 years, PFM has progressed rapidly in its application and theoretical basis. The identification of primary models describing microbial growth curves in both broth and food-based systems has helped this progression (Buchanan, 1993a). For most meat products temperature is the major variable controlling growth rate. Many empirical models have been developed and compared for predicting growth as a function of time for a specific temperature. An early model describing growth rate is shown below (Monod, 1949):

$$N = N_0 \, \mathrm{e}^{kt} \tag{1}$$

Eq. (1) has been used in predicting vacuum ageing periods and shelf-life of refrigerated packaged beef

(Zamora and Zaritzky, 1985), the effects of modified atmosphere on growth of spoilage flora and *L. monocytogenes* on raw chicken (Wimptheimar et al., 1990), and the influence of temperature and pH on the aerobic growth of *L. monocytogenes* on lean beef and fatty tissue (Grau and Vanderlinde, 1992). However the disadvantage of the model lies in the fact that the lag time has to be determined from the data and cannot easily be determined using regression (Schmidt, 1992). Besides the lag phase and the asymptotic value, the maximum-specific growth rate of the growth curve is important. It measures the slope of the growth curve when the organisms grow exponentially. Normally this parameter is estimated by deciding which part of the curve is approximately linear and then determining the slope of this by linear regression (Zwietering et al., 1994). The slope of the part of the curve judged to be linear is a first-order relationship.

Models for microbial growth curves can also be obtained by non-linear regression techniques. Gibson et al. (1987) introduced for the first time in food microbiology the Gompertz function given as follows:

$$y = A + C\exp\{-\exp[-B(t-M)]\} \qquad (2)$$

They then accordingly proposed the use of the following logistic sigmoidal relationship to predict microbial growth:

$$y = \frac{A + C}{\{1 + \exp[-B(t-M)]\}} \qquad (3)$$

In the research (Gibson et al. 1987), the Gompertz and logistic sigmoidal expressions to model the logarithm of counts of *Cl. botulinum* type A in pasteurised pork slurry were compared. The results obtained using the Logistic function were mostly similar to the Gompertz function. However many of the growth curves plotted were asymmetrical. Thus, trying to fit the symmetrical logistic expression was inappropriate. The closer fit of the Gompertz expression was preferred. However Wilcox et al. (1993) showed that growth curves modelled by the Logistic and the Gompertz functions were indistinguishable.

Zwietering et al. (1990) statistically compared several different modified sigmoidal functions (Logistic, Gompertz, Richards, Schnute, and Stannard) using the *t*-test and the *F*-test. The Gompertz

expression was reparameterised to include three biologically relevant parameters: the lag time, specific growth rate and the asymptotic value or maximum bacterial population. The modified Gompertz expression was found to be statistically sufficient to describe the growth data of the test organism, *Lactobacillus plantarum*. In almost all cases, the modified Gompertz expression was regarded as the best model to describe the growth data both in terms of statistical accuracy and ease of use when compared to other sigmoidal functions. Bhaduri et al. (1991) used the modified Gompertz equation to model the non-linear survival curves for *L. monocytogenes* heated in liver sausage slurry. It was concluded that for sigmoidal survival curves it is likely that the equation will give a more accurate prediction of a microbes thermal resistance than a first order kinetic model such as Eq. (1). The modified Gompertz is as follows:

$$y = A \exp\left\{-\exp\left[\frac{\mu_{m} e}{A}(\lambda - t) + 1\right]\right\} \qquad (4)$$

At present, the Gompertz function shown in Eqs. (2) and (4) has become the most widely used sigmoid curve in PFM due to its simplicity and effectiveness (Giannuzzi et al., 1998). It has been used to describe growth curves for many organisms including at least 10 foodborne pathogens. Although the Gompertz function fits growth data well from the lag through exponential to stationary phases of microbial growth, it is not derived from mechanistic considerations. The lack of biological basis for the parameters used makes interpretation of parameters difficult. The calculation of lag time with the Gompertz equation can be wrongly used as growth can occur before the predicted lag time (Marks and Coleman, 1998; Marks et al., 1998). Attempts have been made to replace its empirical nature with more mechanistic-based growth models (Van Impe et al., 1992; Zwietering et al., 1994). Consequently, other models are also available (Baranyi et al., 1993b).

Whiting and Cygnarowicz Provost (1992) proposed a quantitative four-parameter model for the germination, growth and decline of *Cl. botulinum*, and the growth of *L. monocytogenes*. The model was constructed by assuming that spore germination, lag phase or recovery from injury was a first-order process. The models growth rates were found to be 16% less than those derived using the Gompertz

function. Jones and Walker (1993) developed an equation to predict growth, survival and death of microorganisms based on data obtained using *Yersinia enterocolitica* in varying pH and sodium chloride concentrations at different temperatures. In contrast to the Gompertz function, this equation has the capacity to handle growth, survival and death data and it accurately fitted the growth and decline of the organism with lower mean square errors than the Gompertz function. Van Impe et al. (1992) suggested a dynamic first-order differential equation to predict both microbial growth and inactivation, with respect to both time and temperature. This was one of the first models developed to predict microbial growth under dynamically changing conditions. Unlike the Gompertz function, this dynamic model can take the prior history of the food into account. In conditions of constant temperature within the range of growth, the model predictions are the same as the Gompertz function. Later a more detailed framework for describing a microbial population under time varying temperature conditions in a consistent way by using a system theory approach was described (Van Impe et al., 1995).

Baranyi et al. (1993c) proposed a dynamic model for predicting microbial growth, combined with an adjustment function $A(t)$ that depended on the physiological state of the microbial cells (Baranyi et al., 1993a–c). This model is as follows:

$$y(t) = y_{max} - \ln[1 + (e^{-y_{max}-y_o} - 1)e^{\mu_m A_n(t)}] \qquad (5)$$

The value of the adjustment function together with the post-inoculation conditions can predict the duration of the lag phase. With microorganisms of similar pre-inoculation history, the product of the lag phase and the maximum-specific growth rate is a simple change of physiological state. Estimates of this product will then determine how the environmental factors define specific growth rate without the need to model the environmental dependence of the lag phase separately. If the specific growth rate follows environmental variations immediately as they happen, this model can describe microbial growth where parameters such as pH, water activity and temperature change with time, which is found in chilling meat carcasses (Pin and Baranyi, 1998). Many researchers have used the Baranyi model in specific microbial modelling applications and found in com-

parison to the Gompertz function and other models that it gives satisfactory results (McClure et al., 1993; Dalgaard, 1995; Sutherland et al., 1995, 1997; Fernandez et al., 1997; McClure et al., 1997). Primary models have relative advantages and disadvantages but there is a good general agreement between models for microbial growth (Buchanan and Whiting, 1997).

4.2. Modelling the effects of intrinsic and extrinsic conditions on growth within a food matrix (secondary models)

Within food matrix microbial growth kinetics depend on the effects and interactions of intrinsic and extrinsic conditions as listed in Table 1. Manipulation of one or more of these conditions to a level outside the range of growth of most foodborne microorganisms is typically used in preservation techniques. For economic reasons, using mathematical models to predict the effects of and integration of variables almost becomes a pre-requisite for manufacturers. The models used largely depend on numbers of variables and varieties of microorganism involved. Normally a probabilistic or kinetic approach is used. Most probability modelling has been centred on assessing the safety of processed meats with respect to the germination and production of neurotoxins by *Cl. botulinum*. Studies have dealt primarily with the probability of growth of the organism in cured meats. Hauschild (1982) estimated the probability that a single spore would germinate and produce toxin in cured meat products, such as vacuum packaged bacon and liver sausage. The effects of such variables as salt, nitrite, phosphate and sorbate concentration, formulation, processing techniques and pH has also been investigated (Hauschild, 1982; Genigeorgis, 1986; Roberts and Gibson, 1986; Tompkin, 1986; Hauschild, 1989). Some research has been carried out with poultry products (Barbut et al., 1986a,b; Genigeorgis et al., 1991). Probability of growth can be modelled with a logistic probability function and a polynomial equation (Cole et al., 1987):

$$P = \frac{1}{1 + e^{(-n)}} \qquad (6)$$

Useful notes on probability modelling can be found

in literature published by Baker and Genigeorgis (1993), Dodds (1993) and Maas (1993).

Modern approaches to PFM have tried to understand and establish a link between growth of microorganisms and regulatory factors such as temperature. The vast majority of secondary models are kinetically based (Labuza and Fu, 1993), with the most commonly used models being Arrhenius, modified Arrhenius, square-root and polynomial models. Kinetic models for shelf-life prediction are generally based on phenomenon occurring and tend not to be specific for a particular food. However, the experimental and environmental parameters of a model may be fitted with a particular food. Of these temperature is normally considered the most important, as it greatly affects reaction rates. The Arrhenius equation was derived empirically based on thermodynamic considerations (Labuza and Riboh, 1982):

$$k = k_0 e^{\frac{-E_A}{RT}} \tag{7}$$

In Eq. (7) if values of k are recorded at different temperatures and if $\ln k$ is plotted against $1/T$, a straight line is formed with slope $-E_A/R$ (Labuza and Riboh, 1982; Labuza et al., 1992). However bacterial growth is complex and extrapolations of plots may show non-linearity, therefore, Eq. (7) cannot fit data well below optimum or above minimum temperatures for growth. The plots are normally only accurate over a limited temperature range for microbial growth (Labuza and Fu, 1993). Fu et al. (1991) illustrated this accuracy with Arrhenius plots for *Pseudomonas fragi*. Others have also found the equation entirely inadequate in modelling the response variable to temperature in food systems (Ratkowsky et al., 1982; Standard et al., 1985; Phillips and Griffiths, 1987).

Modifications of Eq. (7) have attempted to improve the fitting of this model at temperature extremes. Schoolfield et al. (1981) reparameterised an earlier equation (Sharpe and De Michele, 1977) into a six-parameter non-linear model shown as follows:

$$k(T) =$$

$$\frac{\rho(25°C)\frac{T}{298} \exp\left\{\frac{\Delta H_A^{\neq}}{R}\left(\frac{1}{298} - \frac{1}{T}\right)\right\}}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{\frac{1}{2}L}} - \frac{1}{T}\right)\right] + \exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{\frac{1}{2}H}} - \frac{1}{T}\right)\right]} \tag{8}$$

The temperature of most meat products is critical for ensuring microbial safety in production and distribution. To predict numbers of microorganisms as a function of time and temperature it is necessary to model the lag time, specific growth rate and growth yield as a function of temperature. Zwietering et al. (1991) compared the suitability and usefulness of the Schoolfield model to five other models using *L. plantarum* grown at various temperatures between 6 and 43°C. It was found that the Schoolfield model described data satisfactorily. However, other models such as the Ratkowsky model (Eq. 11) were statistically sufficient, easier to fit and had less parameters.

Davey (1989a) used a modified Arrhenius-type model given below to describe the effects of water activity and temperature on microbial growth rates:

$$\ln k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_w + C_4 a_w^2 \tag{9}$$

Most refrigerated meats would require a significant decrease in moisture content to depress water activity low enough to have an impact on this equation (Labuza and Fu, 1993). Products such as dried or fermented meats, which have depressed water activities may find this equation applicable (Van Gerwen, 1999, personal correspondence). However when water activity is non-limiting the $C_3 a_w$ and $C_4 a_w^2$ terms can be removed (McMeekin et al., 1992). In Eq. (9), all parameters appear linearly and thus estimation can be made using multiple linear regression. The model was applied to published data including that from McMeekin et al. (1987) and Broughall et al. (1983), and subsequently described it well (Davey, 1989a). Duration of the lag phase of microbial growth was also modelled and agreed well with published results (Davey, 1991). Applicability of the model to thermal inactivation of *Cl. botulinum*, thiamine denaturation, aerobic/anaerobic denaturation of ascorbic acid and combined effect of temperature and pH on heat resistance are also described (Davey, 1993a,b). Modified Arrhenius models including the Schoolfield and Davey were formulated to overcome the problems and enhance the original Arrhenius models in fitting data at microbial temperature extremes. Some authors, while describing such models as effective, also point out their complexity and cumbersome nature (Buchanan, 1993a).

The Belehradek model published in 1926 was almost totally unrecognised in microbiology for

many years (Belehradek, 1930; McMeekin et al., 1993; Ross, 1993). Ratkowsky et al. (1982) proposed the first use of the model in food microbiology. The model also known as the square-root model is shown below:

$$\sqrt{k} = b(T - T_{min}) \qquad (10)$$

Ratkowsky illustrated that Eq. (10) satisfactorily described the relationship between microbial growth rate and temperature with over 50 sets of growth data (Ratkowsky et al., 1982) and a further 30 microorganisms in 1983 (Ratkowsky et al., 1991). Pooni and Mead (1984) tested Eq. (10) with other models to data from 14 published studies on poultry spoilage and found that the equation was the most appropriate for predicting spoilage from -2 up to 15°C. The model was later successfully used to model the effects of temperature on bacterial growth rate in meat and raw mutton (Smith, 1985, 1987) and *Salmonella* in minced beef (Mackey and Kerridge, 1988).

Monitoring of meat deterioration due to mesophilic microbial growth was also carried out using temperature integrators programmed with relative rate curves based on mesophilic microorganisms T_{min} values (Gill, 1985; Smith, 1985). The equation was later extended to cover the entire biokinetic temperature range (Ratkowsky et al., 1983). This resulted in a new empirical non-linear regression model shown below:

$$\sqrt{k} = b(T - T_{min})\{1 - \exp[c(T - T_{max})]\} \qquad (11)$$

The terms T_{min} and T_{max} can be used to classify microbes in a more objective manner as psychrophiles, mesophiles or thermophiles (Ross, 1993). Ratkowsky et al. (1983) successfully applied Eq. (11) to data from 29 strains of bacteria. Other researchers have also shown that Eq. (11) is reasonably effective in predicting effects of constant storage temperatures on microbial growth rates (Chandler and McMeekin, 1985a,b; Phillips and Griffiths, 1987; Griffiths and Phillips, 1988; Buchanan and Klawitter, 1992). The effects of fluctuating storage temperature have been studied by Blankenship et al. (1988) who developed a dynamic model for predicting growth of *Cl. perfringens* in cooked meat chilli during chilling using a time-explicit approach.

Various alterations and modifications have been made to Eq. (11). McMeekin et al. (1987) added a

a_w parameter to Eq. (11). It was demonstrated that the model accurately predicted the effect of temperature and a_w on growth rate of *Staphylococcus xylosus* and *Halobacterium* spp., respectively, on salted dried fish (McMeekin et al., 1987; Chandler and McMeekin, 1989a,b). This modified model is as follows:

$$\sqrt{k} = b(T - T_{min})\sqrt{(a_w - a_{wmin})} \qquad (12)$$

However, Chandler and McMeekin (1989a) illustrated that Eq. (12) had no cross-product terms implying that the parameters acted independently of each other. In addition, Eq. (12) could model the growth of *Staphylococcus aureus* without temperature and a_w interaction. Adams et al. (1991) modified Eq. (12) for the combined effects of pH and temperature using a variety of acidulants and showed that growth rate under varying conditions of suboptimal temperature and pH can be predicted using this modified equation which gave good fits for three serotypes of *Y. enterocolitica*. This modified equation is illustrated as follows:

$$\sqrt{k} = b(T - T_{min})\sqrt{(pH - pH_{min})} \qquad (13)$$

Zwietering et al. (1991) made slight alterations to Eq. (13) by first squaring the complete equation and also by squaring just T_{min} to give homogeneous variances and make it more applicable to temperatures above T_{max}. Natural logarithm transformed variations of Eq. (13) have been applied to the growth data of *Y. enterocolitica*. This transformation has been argued to be more reliable than square-root transformation in obtaining homogeneous variances (Alber and Schaffner, 1992, 1993). McMeekin et al. (1992) suggested that the effects of temperature, a_w and pH on microbial growth could be described together with the following equation that was subsequently used successfully on growth data for *L. monocytogenes* by Wijtzes et al. (1993):

$$\sqrt{k} = b(T - T_{min})\sqrt{(a_w - a_{wmin})}\sqrt{(pH - pH_{min})} \qquad (14)$$

Raccach (1992) used Eq. (14) to model the time that *Pediococcus* spp. took to reach a pH of 5–5.3 in fermented meat products. The ability of different cultures of the organism to perform at lower temperatures was reflected in different values for T_{min} and this allowed the prediction of appropriate inoculation levels required to reach a defined pH within a

specific time. The equation has also been successfully used to describe growth data for *L. monocytogenes* (Wijtzes et al., 1993). Modified atmosphere packaging is now commonly used with many fresh meat products. There is a need to evaluate and develop models to examine what effects a change in gas composition will have on microbial growth kinetics. Recently Devlieghere et al. (1999) replaced the pH terms in Eq. (14) with terms to describe for dissolved CO_2 in modified atmosphere cooked meats to examine the significance of this factor on the maximum-specific growth rate of *Lactobacillus sake*. The model proved to be useful in the prediction of the microbial shelf-life of modified atmosphere packed cooked meats.

Many debates have been generated in comparing the relative merits of the Arrhenius and square-root type models. Some have indicated a preference for the square-root model for predicting the effect of temperature on microbial growth (Pooni and Mead, 1984; Standard et al., 1985; McMeekin et al., 1989; Ratkowsky et al., 1991). Fu et al. (1991) concluded that both the square-root and Arrhenius models could fit the lag phase and growth rate of *P. fragi* at constant temperature. In a comparative assessment of eight sets of data for growth of *Cl. botulinum* in cooked turkey, Adair et al. (1989) found that the Schoolfield model was a more reliable description of experimental data. However others indicated that the procedure used by Adair et al. (1989) to compare the models was inappropriate and that the square-root model fitted the data well (McMeekin et al., 1989; Ross, 1993). Baird Parker and Kilsby (1987) found that Schoolfield model was better than the square-root model for predicting growth of *Cl. botulinum* in vacuum-packed minced meat at low temperatures.

The relative complexity of the Schoolfield was highlighted as a non-investigated variable (Davey, 1989b). Kilsby (1989) advocated the use of models showing the best fit to data and thus accuracy over the whole model range must be the most important consideration. Grau and Vanderlinde (1992) demonstrated in fitting the growth data of *L. monocytogenes* on lean and fatty beef by using different versions of the Arrhenius and square-root model, that all models were poor in predicting growth rates and lag times on the fatty samples. Square-root models have been described as the most parsimonious of any models to date achieving parsimony without sacrificing goodness of fit (Ross, 1993). Unfortunately no tests are available for the predictive ability of any of the models (Eqs. (9)–(14)) under conditions where temperature, a_w and pH change with time, as would occur with moisture loss and post mortem pH decline in the cooling of meat carcasses for example (Labuza and Fu, 1993). Interesting and informative discussions of the advantages and disadvantages of secondary models as well as reviews on their development can be found in published literature (McMeekin et al., 1989; Ratkowsky et al., 1991; Baranyi and Roberts, 1992; Ross et al., 1993; Van Boekel, 1996).

5. Development, data collection and validation of models

5.1. Development

In the development of a model, it is essential to know the requirements of the model. Experiments must be designed in such a way as to make the best use of time and resources. Requirements of models can depend on whether the modeller needs to understand the effects of variables on microorganisms or the upper and lower limits for preservation or growth. The inherent variability of both microorganisms and meats means that experiments must be designed to encompass as much of this variability as possible. Many modellers use a mixed culture of the most commonly encountered strains in food for their experiments. In this way growth or survival predicted by the model will correspond to the fastest growing strain present (Ross et al., 1993; Whiting and Buchanan, 1994). A general strategy outlined for experimental design for predictive microbial modelling is given by (Davies, 1993): (i) define experimental objective; (ii) list all variables; (iii) determine the most important variables; (iv) determine the range for these variables; and (v) find microbial optimums or improve understanding.

Development of predictive microbiological models has been reviewed in published literature for *Aeromonas hydrophila* (McClure et al., 1994a), *L. monocytogenes* (Murphy et al., 1996), *Salmonella enteritidis* and *E. coli* O157:H7 (Blackburn et al., 1997), and spoilage *Pseudomonas* (Neumeyer et al., 1997a).

5.2. Data collection

The development of a model requires the generation of data from experimentation. The greater the quantity of data collected, the better the accuracy and reliability of the model derived. To fit primary models and subsequent secondary models, data must be collected over the entire growth period and often over a hundred primary growth curves have to be produced (Bratchell et al., 1989). Multiple variable experiments can take months to carry out (Gibson, 1997, personnel correspondence). Methods of data collection vary among researchers but the standard method is the total viable count. However, the total viable count is a very labour-intensive method. Rapid and automated methods of data collection have been used by many researchers (Cuppers and Smelt, 1993). However, automated methods can have a larger risk of misinterpretation than total viable counts (Krist et al., 1998). How data are generated and recorded is imperative to the practical application and success of a model (Walker and Jones, 1993).

5.3. Validation

Following model development using experimental data, a model must be validated in real situations (McMeekin and Ross, 1996a). This is critical to placing confidence in a model (Whiting, 1997). Validation studies must demonstrate that microorganisms behave in similar ways in both the laboratory and in a real food system. Model validation can be carried out by reference to published results (Blackburn et al., 1997). However this approach can be limited by insufficient or inappropriate data (Williams, 1992). Many modellers use laboratory media to develop and validate models under static conditions (Adair et al., 1989; Hudson and Mott, 1993; Walls et al., 1996). Deviations from predictions using these models are sometimes encountered but do not necessarily imply that a model is defective (McMeekin et al., 1997). Users of specific models must be aware of the boundary of model performance and understand the applicability of the model range. In practice, the issue is not necessarily how well a model fits data, but the accuracy with which it mimics the microbial response (Jones et al., 1994). Some foods can contain variables that have not been validated and this can affect the growth of a specific pathogen. In these cases, a particular model should be evaluated to ensure its sufficient accuracy (Whiting, 1997).

Since variations are common in complex foods (Gill, 1982), many researchers have expressed the need to validate models directly from foods such as meat (Whiting and Masana, 1994; Walls and Scott, 1996). For example, components present in meat product formulations but not in laboratory media result in changes in the environment of microorganisms that could significantly affect the extent to which the environment will support or suppress growth. Sodium nitrite while stable in a laboratory media is rapidly destroyed by reaction with ascorbate, which is present in many commercial meat products (Riordan et al., 1998). In validation, greater emphasis should be placed on the practical use of models. To encourage greater acceptance and reliability from the meat industry will require more independent and industrial-based validation studies under conditions that mimic situations encountered in normal practice such as decreasing temperature and a_w during chilling of meat carcasses (McMeekin et al., 1997).

Validation is often described as an ill-defined aspect of PFM (Ross, 1996). To date no standard methods for model validation have been published. However, Ross (1996) provides some indices of performance for kinetic models to measure their reliability. Validated models should be seen as a summary of a large amount of data representing a general rule, which may be brought to bear on particular cases (McMeekin and Ross, 1996a). Notes on validation can be found in literature published by Walls et al. (1996), Walls and Scott (1996, 1997b), Neumeyer et al. (1997b) and Giffel and Zwietering (1999).

6. Applications of predictive food microbiology

The rapid development of microbial models and their ability to predict microbial growth makes modelling an invaluable research tool. Use of models can quickly provide information and, therefore, it is important to appreciate the real value and usefulness of predictive models. It is also necessary to point out that their applications cannot replace microbial anal-

ysis of samples or the sound technical experience and judgement of a trained microbiologist. Many applications have been proposed for PFM, which will have relevance to the meat industry.

6.1. Hazard analysis critical control point (HACCP)

The acceptance and implementation of HACCP programs in the meat industry requires an ability to deal quantitatively with a range of variables influencing safety (Buchanan and Whiting, 1996). PFM is a quantitative method of describing the effects of these variables on microbial growth, survival or inactivation. As both PFM and HACCP are still being developed as food safety aids, predictive models are available that have potential use in the development and maintenance of HACCP systems (Elliott, 1996). Modelling can help in preliminary hazard analysis, identification and establishment of critical control points, and corrective action to be taken. Interaction between variables such as temperature and a_w is important in application of HACCP in meat processing (Broughall and Brown, 1984). However, it is impractical to determine quantitatively all aspects of microbial growth kinetics in a complex production process in view of the wide range of variables involved. Therefore, a combination of PFM and HACCP offers the meat industry a systematic structured approach of tackling problems, with quantitative calculations when necessary (Zwietering and Hasting, 1997a). PFM can be considered an extension of HACCP (Roberts, 1989; McMeekin et al., 1992).

6.2. Risk assessment

Risk assessment is an analytical tool used increasingly to define priorities for establishing public policy (Buchanan, 1995; Foegeding, 1997). The use of microbial risk assessment in the area of food safety is a newly emerging discipline. Ultimately modellers will need to be able to estimate the possibility whether the consumption of food will cause illness (Anonymous, 1994). However risk assessment is by no means easy. Modelling growth and decline under fluctuating conditions such as

temperature during meat production, processing, storage, distribution and display is complex. To obtain accurate information it is necessary to know the presence and levels of pathogens present, infectious dose, pathogen growth kinetics and quantity of food consumed (Miller et al., 1997). Levels of microorganisms can change at all stages of processing due to multiple interrelated variables. PFM can be used to estimate changes in microbial numbers and to allow exposure to a particular pathogen to be assessed. The characterisation of the disease, dose-response assessment and risk characterisation could also be aided by PFM (Walls and Scott, 1997a).

Risk assessment models of meat production and processing have potential in assisting meat producers, processors, and regulatory organisations in making critical food safety decisions that affect public health (Oscar, 1997). Models have been proposed for *S. aureus* in cooked meats (Walls and Scott, 1997a), *L. monocytogenes* in cooked meatballs (Miller et al., 1997), *E. coli* O157:H7 in ground beef hamburgers (Cassin et al., 1998), *Salmonella* in cooked poultry patties (Whiting, 1997), cooked/chilled food (Buchanan and Whiting, 1996) and cooked chicken (Oscar, 1997, 1998). Oscar (1997) illustrates that the development of a robust risk assessment model for a meat product can be a complex process with many points requiring careful consideration. However, efficiency in quantitative risk assessments results from using simple models. Phenomena that are not quantitatively important should be omitted to prevent over complex risk assessments (Van Gerwen and Zwietering, 1998). The importance of an adequate risk assessment program within the total quality objectives of any company to attain high quality, profitability and safe meat production while observing all the legal requirements is particularly relevant to meat producers (Serra et al., 1999).

6.3. Microbial shelf-life studies

Predictive modelling that integrates microbial behaviour with other process variables has begun to gain interest within the meat industry for predicting shelf-life (Banks, 1994). Shelf-life determination is a complex subject as it is difficult to predict the effects of variable storage and abuse conditions that a product may experience (Williams, 1992). The large

variety and number of spoilage organisms encountered in meat products means that spoilage models are less straightforward to develop than pathogen models and their application is much more limited (Pin and Baranyi, 1998). As with risk assessment and HACCP, shelf-life prediction should consider all stages in the production of a meat product. Accurate data must be obtained about raw materials used, product formulation, product assembly, processing techniques, hygienic conditions, packaging used, storage and distribution procedures and final consumer handling. Only when all these areas are represented will a reliable prediction of shelf-life be possible (Dalgaard, 1995; McMeekin and Ross, 1996b). To follow a shelf-life testing procedure can involve extensive utilisation of both technological and financial resources. However, development of accurate predictive models could in the long term reduce strain on these resources and improve time utilisation (Neumeyer et al., 1997a).

Studies have been carried out on a variety of meat products to determine shelf-life (Vankerschaver et al., 1996; Kant-Muermans et al., 1997; Neumeyer et al., 1997a,b; Devlieghere et al., 1999). However, no studies have used a model capable of incorporating all variables that may have an impact on microbial growth. The main factors influencing microbial stability in meat products are temperature, pH and water activity. Temperature particularly may vary significantly throughout production and distribution (Geeraerd et al., 1998). The majority of studies have used temperature-dependent models, such as the square-root model, and while it is true to say that with most meats temperature is the major factor affecting shelf-life, it is by no means the only variable (Einarsson and Ericksson, 1986; Gill et al., 1988; Gill and Jones, 1992a; Einarsson, 1994). More dynamic models such as those proposed (Van Impe et al., 1992, 1995; Baranyi et al., 1993a, 1996b) are required to accurately predict shelf-life in meat products with more than one fluctuating variable such as temperature. The practical methods used in predictive modelling for shelf-life need to advance. Standard microbiological methods of analysis although effective are slow. Future research should take into account the short shelf-life of much chilled meat and the fact that results are required quickly (Gibbs and Williams, 1990). Use of more rapid microbiological techniques will be required.

6.4. Temperature function integration and meat hygiene regulatory activity

The consequences for hygiene and safety of perishable products during their short shelf-life can be estimated or predicted using temperature function integration (TFI). This technique uses the prior temperature history of the product and integrates this with the temperature-related characteristics of specific microorganisms. Typically TFI can be applied to food storage, cooling, distribution or display (Gill, 1996). TFI application in assessing meat cooling processes has been reviewed (Gill et al., 1991a,b; Gill and Jones, 1992a,b, Reichel et al., 1991). Lowry et al. (1989) considered TFI application in determining hygienic efficiency of meat thawing operations. Skinner and Larkin (1998) illustrated how integrating-type time-temperature indicators can warn food processors and consumers about storage conditions that may have rendered a food potentially hazardous to *Cl. botulinum* toxin formation. The use of TFI has been found to be rapid and cost effective in quantifying a temperature-dependent process in terms of potential for microbial growth. The incorporation of predictive models into temperature logging devices has been illustrated for both *E. coli* and *Pseudomonas* in meats were the temperature data can be used to interpret microbial growth (Gill and Phillips, 1990; McMeekin and Ross, 1996a). However, any interpretation must be based on an informed analysis of the data by a trained operator (McMeekin et al., 1997).

6.5. Product research and development

Altering a product composition or processing regime can have significant effects on the microbial population or opportunistic microbial growth. PFM can provide a means to quickly evaluate the consequences of any changes in formulation or processing. However, it cannot avoid but can reduce the need for expensive, time consuming challenge tests. Problems in production of existing products can also be evaluated in terms of out-of-specification circumstances. Modelling can help a manufacturer to quickly decide on whether to use, destroy, rework or put a product on hold for suitable analysis (Blankenship et al., 1988; Buchanan et al., 1989; Roberts, 1990). The incorporation of HACCP in the initial stages of

product development allows for assessment of the severity of risk from raw materials used in their processing, distribution and intended use. Predictive microbial models can become an integral tool to evaluate control, document and defend safety design in a new or existing product (Baker, 1995).

6.6. Education

PFM offers a front seat view on the behaviour of microorganisms in foods in response to changes in intrinsic and extrinsic variables. Laboratory experiments tend to be time consuming, expensive and often not very illustrative. However models of microbial behaviour using graphs or estimates of time to a specified microbial level can clearly show responses (Whiting, 1995). This is especially useful in the education of non-technical people. Models can interactively demonstrate to individuals the need to do their jobs correctly and the consequences if this goes wrong (Walker and Jones, 1992). The role of PFM in educating both technical and non technical personnel is equal. Although many technical people would be familiar with microbial responses to change, clear visual representation of these changes can often reinforce their existing knowledge and help them apply it more productively. Graph generation through modelling can clearly illustrate the importance of critical control points in a HACCP program. This in turn can help a manufacturer to create a more sophisticated and effective HACCP program in a multi-step food processing operation (Whiting and Buchanan, 1994).

6.7. Other applications

Within the laboratory environment, models can be used to quickly give the ranges of concern for a variable and thus allow better design of experiments. Selection of specific conditions for enrichment of target organisms in the laboratory could also be maximised (McMeekin and Ross, 1996a). In the US the USDA/ARS/ERRC's Microbial Food Safety Research Unit laboratory uses models to devise laboratory work schedules for sampling timed experiments and analysing microbial data (Whiting, 1997). McMeekin and Ross (1996a) applied predictive modelling to describe the effect of environmental variables on microorganisms deliberately added to

foods including meat products to produce a desired effect. Breidt and Fleming (1998) addressed the issue of modelling the competitive growth of pathogens such as *L. monocytogenes* in mixed cultures of bacteria. The research may aid in the selection of lactic acid bacteria for use in competitive inhibition of pathogens in minimally processed fermented meats. Pin and Baranyi (1998) used predictive modelling to quantify the concepts of dominance and influence of major spoilage organisms found in aerobically stored refrigerated meat under the influence of temperature and pH.

7. Further research in predictive microbiology

As the criteria for most meat products shelf-life is set by spoilage, mathematical models to predict for the major groups of spoilage microorganisms would be useful. The development of mathematical models to predict microbial spoilage of foods is normally very product and/or industry specific. A generic model developed for a specific spoilage organism, therefore, may have limited appeal. Development of comprehensive models for spoilage organisms has not received much attention. However in recent years research into predicting spoilage has gained much interest (Zwietering et al., 1992; Gibson et al., 1994; Cuppers et al., 1997; Pin and Baranyi, 1998; Olvera et al., 1999). McMeekin and Ross (1996b) illustrated that to date most spoilage models deal with fish and dairy products, with few dealing with meat particularly processed meats. Some models have been proposed to describe growth of starter cultures in fermented sausages (Bello and Sanchez-Fuertes, 1995; DoBmann et al., 1996). Nevertheless, only the model of Aggelis et al. (1998) for example is available to predict microbial growth in a raw cured meat product.

In terms of growth properties, most models to date refer to predicting growth rate or generation time. The relevance of such a model may be questioned, as with most pathogens any growth in a product is unacceptable. A greater understanding of the lag phase of microbial growth and the physiological affecting factors is needed. There is also a need to predict lag time accurately. Attempts have been made for many organisms in predicting lag times (Buchanan and Cygnarowicz, 1990; Genigeorgis et

al., 1991; Zwietering et al., 1991; Van Impe et al., 1992; Baranyi et al., 1993a; Breand et al., 1997; McKellar et al., 1997). However, lag time predictions have shown much less reliability than growth rate predictions (Zwietering and Hasting, 1997a; Robinson et al., 1998). One problem in predicting microbial growth under changing conditions such as temperature is that changes in the physicochemical environment can result in a growth lag phase, in which the microbial cells adapt to their new conditions. Any practically useful approach to modelling must, therefore, be able to take account of lag phase under different or fluctuating conditions, which are prevalent in many meat products.

There is also a need for increased metabolic research to understand microbial physiology so that primary and secondary models can have a platform based upon the intrinsic and extrinsic physiological, chemical and physical interactions of microorganisms. This can lead to greater understanding of why certain microorganisms are more or less tolerant to environmental conditions (Ko et al., 1994; Smith, 1996; Stecchini et al., 1998).

The emergence of low-infectious dose pathogens particularly with ground beef products presents a significant challenge to predictive microbiology (Miller et al., 1998). In cooking ground beef products rate of decline of *E. coli* 0157:H7 will depend on prior storage temperatures and the fat content of the product (Jackson et al., 1997). Some research has indicated that increasing fat content of meat provides protection to pathogens during cooking (Line et al., 1991, Ahmed et al., 1995). Predictive models need to consider how the thickness of product such as burgers will affect pathogen survival. Thicker burgers will provide a greater probability of pathogen survival if cooking is inadequate. Incorporation of heat transfer equations into models could help determine temperature changes during all stages of storage and preparation (Alavi et al., 1996; Juneja et al., 1997). Greater emphasis needs to be placed on modelling the death kinetics of these pathogens so that effective models can be developed for incorporation into HACCP and risk assessment systems in meat production.

Competition between microorganisms in foods is not considered in most predictive models. The growth of a pathogen in ground beef for example is dependent on both its initial population density and that of competing organisms (Coleman et al., 1996). Growth of pathogens can be affected by production of bacteriostatic microbial by-products such as bacteriocins. In Europe a purified bacteriocin called Nisin is already in use in some meat products to prevent microbial growth. Recently, other researchers have begun to address the area and develop models to predict the effect of competitive growth on pathogens (Breidt and Fleming, 1998; Pin and Baranyi, 1998).

Finally, there is a necessity to link a series of models together from each step in a food process chain and hence provide a risk analysis of the operation. Research in risk assessment is ongoing within the scientific community. However, current uncertainty and lack of knowledge about the effects of particular operations during production, processing and distribution of food has meant that practical application of risk assessment in industry has being limited. Continuing research, which integrates risk assessment with PFM and analysis of a particular situation, could help in the objective and accurate assessment of a production process. The development of sophisticated nonthermal preservation techniques in the meat industry such as high hydrostatic pressure processing, high intensity pulsed electric field processing, oscillating magnetic field processing, light pulse sterilisation and food irradiation could have significant effects on microbial physiology, that in turn will affect the ability to produce accurate predictions (Barbosa-Canovas et al., 1998). As modelling moves towards more mechanistic approaches such as connecting behaviour of a single cell to that of a whole population in making predictions, so more variables may need to be considered (Baranyi, 1997). However, overparameterisation can make models unnecessarily complex. Thus, models should take into consideration only those variables that impact significantly on microbial responses. This may require greater generation of product specific models.

8. Conclusions

The role of PFM has been increasing over the last decade. Research teams working throughout the world have made many advances in developing and implementing models based on extensive data collec-

tion. However extensive research still remains if predictive food microbiological techniques are to be fully accepted.

Classification of models still shows a lack of clarification with no scheme having the full support of the scientific community. However the proposed three-tier scheme of primary, secondary and tertiary models is an advance to greater clarity. Availability of many different models can make selection of the best model for a particular use difficult. In specific situations some data can work better with one model than another. However using the simplest available model without compromising too much on accuracy is a rational policy.

Development of more accurate and sophisticated models is ongoing. Models incorporating more intrinsic and extrinsic variables coupled with improved understanding of microbial physiology are a higher level of development. The use of PFM in the development of quantitative microbial risk assessments is an emerging application with big implications for industrial safety, economics and public health. Application of most existing growth models to meats is appropriate. However in some ways they may be better for cooked meats, as the majority of spoilage flora is gone.

The steady and careful implementation of predictive microbiological techniques into academic institutes and industry is critical to its continued use and acceptance. Errors made using such techniques in relation to industry and public health could have detrimental effects on the future research and development of PFM. Its use in real situations should not be applied unless a defined level accuracy and validation is achieved. The reliance of food microbiology on laboratory techniques to determine process and food safety is still necessary. However, what is apparent is that PFM will continue to increase in importance within food microbiology as we move towards the 21st century.

9. Nomenclature

A	asymptotic log count as t decreases indefinitely $(CFU)^{-1}$
$A(t)$	precise integral of the adjustment factor
b	regression coefficient for temperatures below optimal (K^{-1})
B	relative growth rate at time M (s^{-1})
c	regression coefficient for temperatures above optimal (K^{-1})
C	asymptotic amount of growth that occurs as t increases indefinitely log $(CFU)^{-1}$
$C_0 - C_4$	coefficients
E_A	activation energy $(J\ mol^{-1})$
$\Delta H^{\neq}_A$	enthalpy of activation of reaction catalysed by enzyme $(J\ mol^{-1})$
ΔH_H	change in enthalpy associated with high temperature inactivation of enzyme $(J\ mol^{-1})$
ΔH_L	change in enthalpy associated with low temperature inactivation of enzyme $(J\ mol^{-1})$
k	growth rate constant (s^{-1})
k_o	Arrhenius equation constant (pre-exponential factor)
M	time at which absolute growth rate at maximum (s)
n	fitting curve parameter
N	number of organisms at time t $[(CFU)^{-1}]$
N_o	initial number of organisms at time $t = 0$
P	probability
pH_{min}	minimum pH for growth
R	universal gas constant (8.314 J mol^{-1} K^{-1})
t	time (s)
T	temperature (K)
T_{max}	notional maximum growth temperature (K)
T_{min}	notional minimum growth temperature (K)
$T_{\frac{1}{2}H}$	temperature at which the enzyme is 50% inactive due to high temperature (K)
$T_{\frac{1}{2}L}$	temperature at which the enzyme is 50% inactive due to low temperature (K)
x	polynomial
y	logarithm of relative population size
Y_o	logarithm of initial population size
Y_{max}	logarithm of maximum population size
a_w	water activity
a_{wmin}	minimum water activity for microbial growth
λ	lag phase duration (s^{-1})
μ_m	maximum-specific growth rate (s^{-1})
$\rho(25°C)$	development rate at 25°C assuming no enzyme inactivation (s^{-1})

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