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ORIGINAL ARTICLE

Application of Food MicroModel predictive software in the development of Hazard Analysis Critical Control Point (HACCP) systems

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A practical application of the Food MicroModel (FMM) predictive software is presented. A case study on meat-based pâté is used to illustrate the various requirements needed to assure the safety of this type of foodstuff when pH is reduced. Identification of hazards was obtained from a literature review and confirmed by epidemiological links between the product and foodborne disease outbreaks. For risk assessment four different zones (safe, caution, dangerous and critical) of the level of the variable under study (pH) were defined, each zone equating to a particular level of risk. Having identified the hazards, associated risks and intrinsic parameters of the pâté, a Hazard Analysis Critical Control Point (HACCP) system can be more readily established using predicted outcomes from FMM. General guidance on generic uses is also discussed.

Introduction

Microbial risk assessment is becoming a crucial procedure in the safety of foods. It is important, from a legal point of view, since companies need to produce food according to the requirements of the 1995 Food Safety (General Food Hygiene) Regulations which require the use of risk assessment techniques to ensure the safety of the products (Jacob 1996). The very nature of foods inevitably leads to some level of contamination at some point which means that although the hazard may be present, the risk, the probability that an adverse effect will occur (Notermans et al. 1996), of illness related to that hazard may not necessarily be great. Therefore the new task of the food industry is to maintain the level of risk at a minimum that is practical and technologically feasible (WHO 1995).

Within Hazard Analysis Critical Control Point (HACCP) plans, risk assessment involves two basic components, the identification and the assessment of hazards (Baird-Parker 1994). Identification requires a literature review of likely pathogens, surveys of the microbial composition of raw materials (Notermans et al. 1994, Baird-Parker 1994) and epidemiological data on the surveillance of foodborne infections and intoxications (Buchanan et al. 1993, Notermans et al. 1994, Potter 1994, Weingold et al. 1994, Baird-

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Parker 1994, Buchanan 1995). Once hazards have been identified, a precise data of their presence in raw products can be obtained, but further information will be necessary to determine which hazards will be present at the point of consumption. The latter requires an assessment of the impact of intrinsic, extrinsic and other preservative factors used during production, distribution and retail on the growth of survival of identified hazards (Baird-Parker 1994, Buchanan 1995) and, more importantly, which associated risk is or is not acceptable (Notermans et al. 1995).

The interaction between the different controlling factors that affect bacterial growth and the probability that a given micro-organism will grow, survive or die under these conditions can be studied by use of microbiological challenge testing (MCT), storage testing (ST) and, more recently, predictive microbiology instruments. The former two methods are slow and expensive procedures (Roberts 1996a) and applicable to a reduced set of conditions where results can be relied on (Notermans et al. 1994, Notermans et al. 1995). They lack an important predictive component in assessing the changes in product formulation, processing or packaging. Thus, predictive models can be readily used to evaluate the changes of a wide range of factors upon the growth of the micro-organisms of interest (Baird-Parker 1994, Notermans et al. 1995). Hence predictions anticipate the event of bacterial growth helping the decision-making process (McClure et al. 1994).

Predictive microbiology often involves the use of mathematical formulae which best describe microbial behaviour (Whiting and Buchanan 1994) and only comparatively large organizations have been able to benefit from their use. However, in the late 1980s the Ministry of Agriculture, Food and Fisheries (MAFF) identified predictive microbiology as one approach to help ensure food safety (McClure et al. 1994); this triggered the development of an ambitious, co-ordinated programme of research which resulted in a computerized predictive microbiology database known as Food MicroModel (FMM).

This paper describes the role of FMM as a supporting instrument for microbial risk assessment in the HACCP system using a meat product, pâté, as an example. The effect of lowering the pH of pâté is used to illustrate the advantage of modelling in assessing risks, product formulation variations, helping the decision-making as to whether or not the product is safe, as well as HACCP development.

Materials and Methods

Hazard identification

Microbial hazards were identified first (presumptive analysis) using microbial surveys on the microbial quality of pâté and/or related products from a wide range of literature sources. Secondly, a confirmatory analysis was carried out on the preselected pathogens which were evaluated on the basis of epidemiological evidence of their involvement in foodborne disease outbreaks. This information was obtained from literature sources including communicable disease reports published by the Public Health Laboratory Service.

Risk assessment

Defining growth parameters. This involved identifying the critical factors likely to affect the growth of the pathogens in the food product. The variable factor was pH and the stable parameters were temperature (fixed at 8°C for the purposes of the analysis) and NaCl content (fixed at 0·5% based on typical formulations).

All predictions were made using the FMM software package, version 2·52. FMM was used to determine (a) the growth rates for the pathogens across a range of pH values, (b) predicted lag phases for the pathogens under the same conditions and (c) log cfus (colony-forming units) obtained after 7 days at various pHs. In order to compare different micro-organisms it was assumed that $10\ \text{cfu g}^{-1}$ survived the processing treatments (which is the minimum value accepted

by the model) and that the product was kept at refrigerated storage temperature of 8°C which is the minimum legal requirement. For *Escherichia coli* O157:H7, the growth model was run at 10°C, the minimum within the program's range. With respect to the food parameters, previous studies defined the pH of pâté as between 6·1 (Hudson and Mott 1993b) and 6·8 for Ardenne-type pâté (Tassou et al. 1995). Such differences are attributable to the different compositions of ingredients and preservatives for this product. A value of 0·5% NaCl (w/w) was similarly identified as typical of pâté. Predictions were obtained by reducing the pH value from 6·8 to the minimum pH value for the program's limits for each pathogen (Table 1).

Related to this, there was initially a need to attempt to have an objective basis for risk assessment based on growth/no growth basis only. This was achieved by defining four zones that related to differing levels of safety associated with the product which were defined based on various pH values as affecting microbial growth according to FMM. These zones are defined as follows:

- *Safe zone*: comprises a range of pH values at which, according to the experimental limits of the model, no growth is predicted. No risk is associated within this zone.
- *Caution zone*: comprises a range of pH values wherein growth is recorded but the lag phase exceeds the shelf life of the product (which was considered to be 7 days). Risk remains at a minimum within this zone and the food will be safe if consumed before 7 days. Control should be exercised to keep product formulation conditions between the limits of this zone.
- *Danger zone*: comprises a range of pH values wherein growth is recorded and the lag phase does not exceed the shelf life of the product (7 days in the present case). The associated risk becomes so high that product safety cannot be guaranteed unless some of the conditions are modified. Control is required to change conditions to a safer zone.
- *Critical zone*: comprises a small range of pH values within the danger zone, where the bacteria grow at a maximum rate. The associated risk reaches its maximum, indicating that control needs to be exercised to avoid this zone.

Thermal abuse. The effect of thermal abuse was modelled using the same controlling parameters for each pathogen, a pH of 6·8 and a temperature of 22°C (except for *Yersinia enterocolitica* for which the temperature was 15°C owing to the limitations of

Table 1. Food MicroModel controlling growth factors used for each model

		Controlling factors				
		Fixed			Variable	
Pathogen[a]	Model type	Temp (°C)	NaCl (% w/w aq)	a_w	pH High	pH Low
Salmonella spp.	G&S[d]	8	0·5	0·977	6·8	3·9
Aeromonas hydrophila	G&S	8	0·5	0·977	6·8	4·6
Listeria monocytogenes[b]	G&S	8	0·5	0·977	6·8	4·4
Yersinia enterocolitica[b]	G&S	8	0·5	0·977	6·8	4·2
Clostridium botulinum[c]	G&S	8	0·5	0·977	6·8	5·0
Staphylococcus aureus	G&S	8	0·5	0·977	6·8	4·2
Escherichia coli O157:H7	G&S	10	0·5	0·977	6·8	4·5
Campylobacter jejuni	S[e]	8	0·5	0·977	6·8	4·3

[a] Initial inoculum = 1 $\log_{10}$ (cfu g^{-1}).
[b] Lactic models.
[c] Non-proteolytic model.
[d] Growth and survival model.
[e] Survival model.

the model) and predicting the log cfu g^{-1} after 5, 8, and 10 h.

Results

Hazard identification

Presumptive analysis was based on primary and secondary sources of contamination which, according to the literature, can potentially be associated with pâté. Identification of primary sources of micro-organisms includes an evaluation of the main raw ingredients such as red meats, poultry and its offal (Tompkin 1993, Mead 1994), which can become contaminated with several pathogens during slaughtering practices, for example, *Y. enterocolitica* (ICMSF 1996, Kapperud 1991), *Salmonella* spp. (Oosterom 1991, ICMSF 1996), *Campylobacter* spp. (Skirrow 1991, Pearson and Healing 1992, ACMSF 1993, Pebody et al. 1997) and *E. coli* O157:H7 (ACMSF 1995). Secondary sources of micro-organisms can be obtained by the use of microbiological surveys on the microbial quality of the finished product (pâté) and related products (cook–chill and delicatessen products). Direct relations were found for *Aeromonas hydrophila* (Hudson and Mott 1993a) and *Listeria monocytogenes* (McLauchlin et al. 1991, Gilbert et al. 1993, Gilbert 1996) in delicatessen samples and pâté respectively. Toxin-forming pathogens were also reviewed. *Clostridium perfringens* is a primary source of contamination since it can be found just after slaughter in deep muscle and liver (ICMSF 1996). *Cl. botulinum* spores can be found in meats but at a very low contamination level (Gaze 1992, Dodds 1994). Psychrotrophic strains of *Cl. botulinum* are a recognized hazard in chilled vacuum-packed, ready-to-eat foods which usually have minimal heat processing and low preservative content (Schofield 1992). Contamination of carcasses when poor hygienic post-processing operations are practised is the probable route of contamination of poultry and other raw meats with *Staphylococcus aureus* (Martin and Myers 1994).

Confirmatory analysis was based on epidemiological evidence of a given product as a vehicle of foodborne disease outbreaks. This was 'direct' when searching for the product itself, or 'indirect' when searching for related products. *L. monocytogenes* was reported in 1988 to be directly linked to pâté (McLauchlin et al. 1991, Gilbert et al. 1993). Indirect analysis established a link between *Campylobacter* and chicken liver mousse (Pebody et al. 1997). Furthermore, raw meats and meat products contaminated with *Salmonella* spp. (Oosterom 1991), *Campylobacter* spp. (ACMSF 1993, Sockett et al. 1993), *Y. enterocolitica* (Kapperud 1991), *E. coli* O157 (Sockett et al. 1993, ACMSF 1995), *Cl. perfringens* and *Staph. aureus* (Sockett et al. 1993, Djuretic et al. 1996) have been reported to act as food vehicles in several foodborne outbreaks worldwide (Roberts 1982, Bryan 1988). Although aeromonads can be present in pâté or related products definitive links between foodborne *Aeromonas* spp. and illness could not be established, probably because of the sporadic and rare nature of foodborne episodes (Eley 1996). However, since *Aeromonas* spp. can contaminate processing equipment in slaughtering plants (Gill and Jones 1995) as well as the environment of supermarket delicatessen (Hudson and Mott 1993a) it was identified as a potentially hazardous organism for pâté. *Cl. perfringens* was not considered for further study since the temperature used (8°C) was outside the limits of the model. Therefore challenge testing on the survival of spores as well as consideration of possibility of germination when pâté is thermally abused is advisable.

Predictive models versus pH

The predictive models were used as described in the **Materials and Methods** section to establish possibilities for growth and survival of the selected pathogens and to establish the four zones described above in order to produce an objective basis for the initial risk assessment.

Infectious species: Aeromonas hydrophila. Growth characteristics: Predicted

growth of *A. hydrophila* was recorded between pH 4·9 and 6·8 (Fig. 1(a)). Small increases in growth rate were predicted in pH values below 4·9 (Fig. 1(a)), but there was no associated predicted increase in numbers after 7 days within this pH range (Fig. 3(a)). The lag phase was predicted to be 23 days, the longest for all the infecting pathogens examined (Fig. 2(a)).

Risk assessment zones: Since no growth of *A. hydrophila* was predicted at pH 4·9 or below, a safe zone of pH 4·9 or below could be defined. Lag phases of longer than 7 days were noted at between pH 4·9 and 5·0 which determines the caution zone for the microorganism. The danger and critical zones of 5·1–6·8 and 6·7–6·8 are easily defined from the predictions obtained. This narrow caution zone is clearly a concern because control of parameters within such narrow limits in a manufacturing situation may be difficult to achieve (Table 2).

Listeria monocytogenes. Growth characteristics: Predictions for *L. monocytogenes* showed growth between pH 4·4 and 6·8 (Fig. 1(a)) and a maximum recorded lag phase of 10 days was observed at pH 4·4 (Fig. 2(a)). Increases in numbers of organisms predicted after 7 days climbed steeply up to a pH of 6·2, with almost a plateau being obtained thereafter with a value of $\log \text{cfu g}^{-1} = 5{\cdot}9$ being predicted at pH 6·2 and a corresponding value of 6·6 at pH 6·8 (Fig. 3(a)). Predictions at low pH values did not show arrested growth of the pathogen, which represents a cause for concern.

Risk assessment zones: A safe zone of less than pH 4·4 was established but, since this was below the limit of the model, it should again be regarded as presumptive. The caution zone was set between pH 4·4 and 4·6. A danger zone of pH 4·7 to 6·8 suggests that this organism is unlikely to be controlled adequately within the investigating parameters by pH reduction, which is cause for concern. (Table 2).

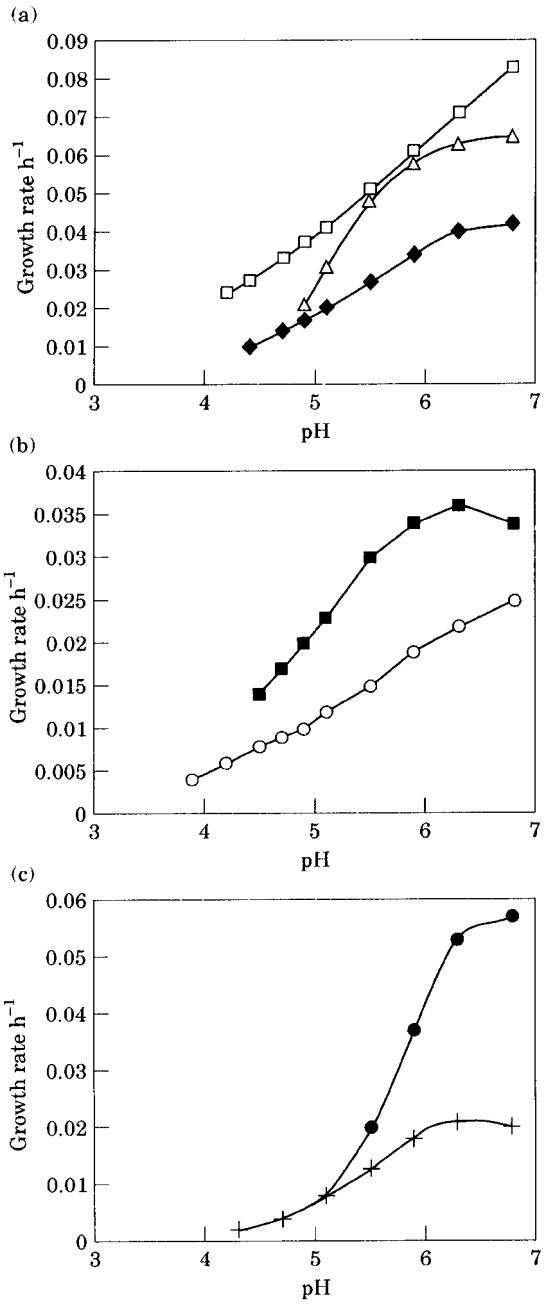

Figure 1. Predicted growth/survival rate ($\log_{10}$ growth h^{-1}) of: (a) *Aeromonas hydrophila* (△), *Yersinia enterocolitica* (□), and *Listeria monocytogenes* (◆); (b) *Salmonella* spp. (○), *Escherichia coli* O157:H7 (■); and (c) *Clostridium botulinum* (●), *Staphylococcus aureus* (+) present in pâté vs pH at 8°C.

Yersinia enterocolitica. Growth characteristics: Predictions for *Y. enterocolitica*

showed the highest growth rates for all infective types and growth was predicted in the range of pH 4·2 to 6·8 (Fig. 1(a)). Reducing the pH, however, had a marked effect on growth but growth was still observed at pH 4·2 (0·024 $\log_{10}$ increase in cell numbers h^{-1}). *Yersinia* displayed the lowest predicted lag times overall for the infective pathogens with values of 4·6 and 0·7 days at pH values of 4·2 and 6·8, respectively. *Y. enterocolitica* also displays the highest predicted number of log cfu g^{-1} at 7 days, pH 6·8, for all the organisms examined (8·4 log cfu g^{-1}) (Fig. 3(a)). In view of the association of this organism with meat and meat products, its ability to reach such comparatively high numbers at 8°C should be treated with caution.

Risk assessment zones: A lag phase of 4·6 days was observed at pH 4·2, which made it impossible to establish a caution zone for the organism (Fig. 2(a)). Also, the limit for the zone is presumptive in that pH 4·2 is the lower limit of the model and so growth would need to be confirmed by microbial challenge testing (Table 2) in view of the data described above relating to food associations of the organism. Critical and danger zones for the organism were established, as shown in Table 2. Predictions at low pH values did not indicate arrested growth of the pathogen, which gives cause for concern, as has been described for *L. monocytogenes*.

Salmonella *spp.* Growth characteristics: *Salmonella* spp. showed the widest pH growth range of the infective pathogens tested with a lower limit of pH 3·9 and an upper limit of 6·8 (Fig. 1(b)). The predicted growth rate of *Salmonella* was the lowest of all infecting types, however, with a value of 0·025 $\log_{10}$ increase in cell numbers h^{-1} being observed at pH 6·8. The number of cells was also comparatively small, which relates to the low growth rate (Fig. 3(b)). A lag phase of 6 days was observed at a pH of 3·9 (Fig. 2(b)) which is one of the lowest for the organisms analysed. However, it should be noted that predicted growth in these conditions (pH 3·9 and 8°C) must be confirmed with experimental data.

Risk assessment zones: A safe zone was established as less than pH 3·9, since this was below the limits of the model and so is best described as presumptive. However, most literature sources would support the view that this pH represents a minimum for *Salmonella* spp. (ICMSF 1996). It was not possible to establish a caution zone for the organism since a lag phase of 6 days was observed at a pH of 3·9 (Fig. 2(b)) and a small increase in numbers from $\log_{10}$ 1 to 1·3 in 7 days was observed at the same pH. This relates to a doubling time of 68·4 h predicted at pH 3·9 from the model. Danger and critical zones, however, could be defined (Table 2) and relate to a lag phase of less than 7 days

Table 2. Calculated pH zones for pâté based on Food MicroModel outcomes at 0·5% NaCl (w/w) and 8°C for different pathogens

Pathogen	Safe zone	Caution zone	Dangerous zone	Critical zone
Salmonella spp.	<3·9[a]	–	3·9–6·8	6·7–6·8
Aeromonas hydrophila	<4·9	4·91–5·0	5·1–6·8	6·7–6·8
Listeria monocytogenes[b]	<4·4	4·4–4·6	4·61–6·8	6·4–6·6
Yersinia enterocolitica[b]	<4·2[a]	–	4·21–6·8	6·6–6·8
Clostridium botulinum[c]	<5·5	5·6–5·7	5·71–6·8	6·6–6·7
Staphylococcus aureus	<4·3	4·31–5·0	5·01–6·8	6·3–6·7
Escherichia coli O157:H7[d]	<4·5[a]	–	4·5–6·8	6·1–6·5
Campylobacter jejuni[e]	5·5	5·5–6·8	–	–

[a] Presumptive lower limit based on model data.
[b] Lactic models.
[c] Non-proteolytic model.
[d] Growth model was run at 10°C.
[e] Survival model.

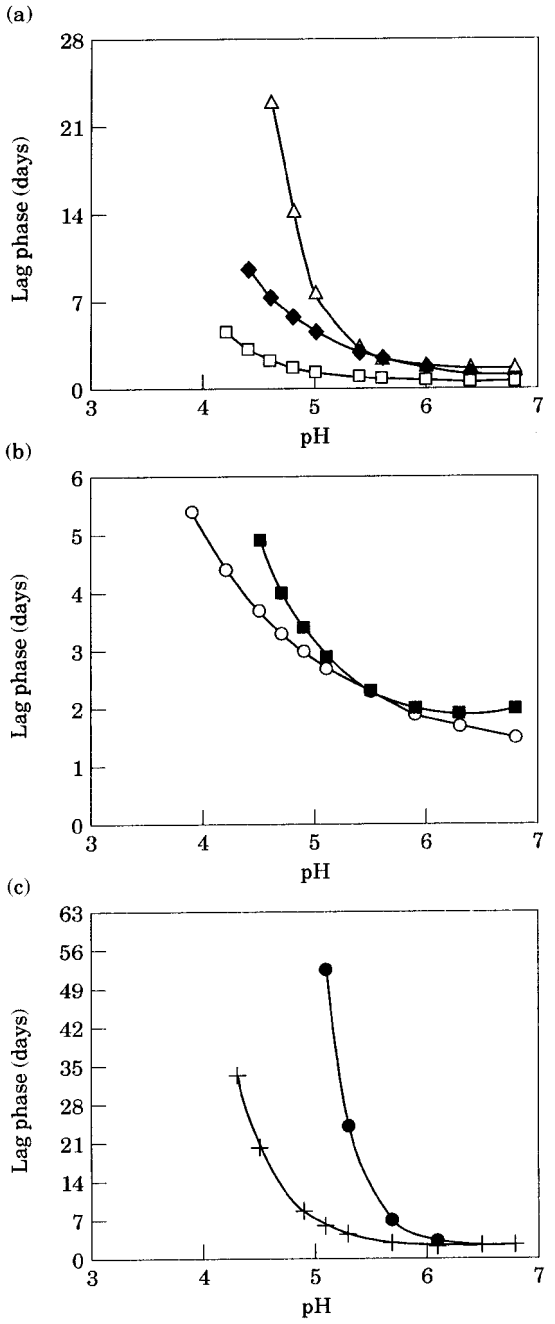

Figure 2. Predicted lag phase (days) of: (a) *Aeromonas hydrophila* (△), *Yersinia enterocolitica* (□), and *Listeria monocytogenes* (♦); (b) *Salmonella* spp. (○), *Escherichia coli* O157:H7 (■); and (c) *Clostridium botulinum* (●), *Staphylococcus aureus* (+) present in pâté vs pH at 8°C.

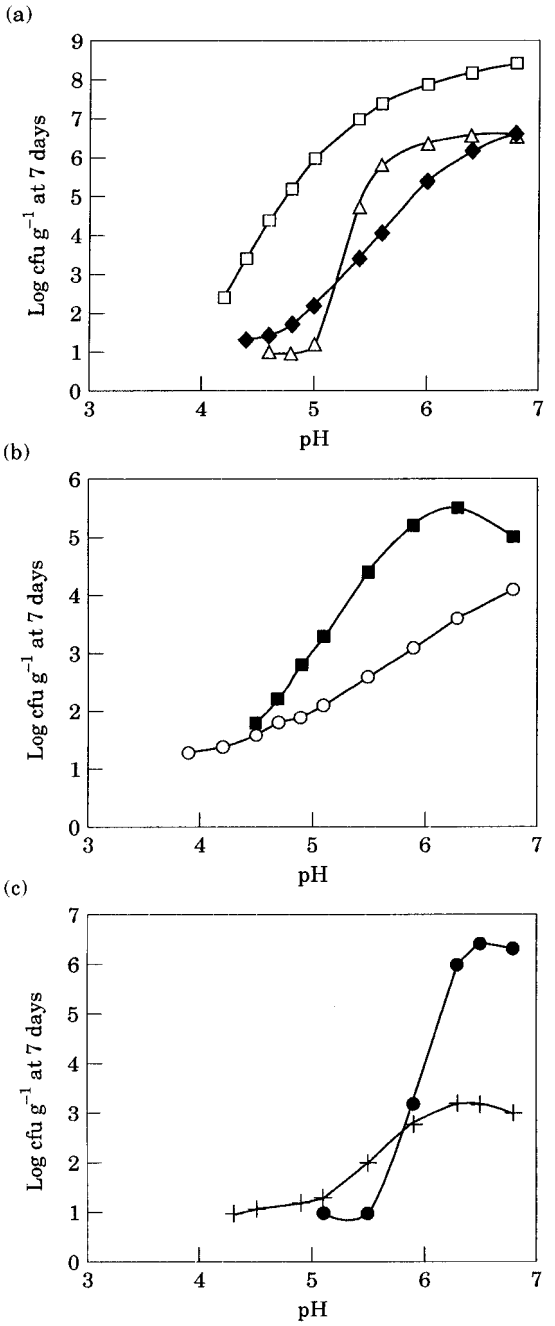

Figure 3. Predicted number of cells (log cfu g^{-1}) of: (a) *Aeromonas hydrophila* (△), *Yersinia enterocolitica* (□), and *Listeria monocytogenes* (♦); (b) *Salmonella* spp. (○), *Escherichia coli* O157:H7 (■); and (c) *Clostridium botulinum* (●), *Staphylococcus aureus* (+) present in pâté vs pH at 8°C.

and pH values at which the predicted growth rate is at its maximum.

Escherichia coli O157:H7. Growth characteristics: The overall predictions of growth

of *E. coli* O157 were from pH 4·5 to 6·8 (Fig. 1(b)). The growth rate at pH 4·5 was 0·015 $\log_{10}$ increase in cell numbers h^{-1} which is the highest rate for this group and comparable to that of *Y. enterocolitica* (bearing in mind that the *E. coli* O157 model was run at 10°C). This underlines observations on the acid tolerance of *E. coli* O157 and its significance in foodborne disease outbreaks (Miller and Kaspar 1994). The lag phase was overall the shortest of this group, allowing for the temperature at which the model was run (Fig. 2(b)). The data presented in Fig. 3(b) indicate that *E. coli* O157 was the least pH dependent of the organisms in this group, which is of significance in pH-controlled foods. However, the results suggest that *E. coli* O157 has the potential to create problems in this type of food product even if the pH is reduced below pH 6·1.

Risk assessment zones: A presumptive value of below pH 4·5 was established as the safe zone which in view of the reported acid tolerance, particularly of *E. coli* O157:H7, should be confirmed experimentally. A caution zone could not be established because of the predicted lag phases of the organism. The widest danger zone of this group of pathogens was set from pH 4·5 to 6·8 with a critical zone between pH 6·1 and 6·5 based on predicted growth rates (see Table 2).

Campylobacter jejuni. This microorganism differs from the others in this group in that it cannot multiply in foods, although it may possibly survive depending on environmental conditions (Pearson and Healing 1992). *C. jejuni* survived poorly because of a combination of pH and low temperature, with poorest survival (fastest decline rates) being observed at pH values of less than 5·4. This effectively sets the limit between the safe and caution zones (Table 2). Danger and critical zones are not possible to set from the predictions given because of the need to use the 'survival model' of FMM.

Toxin-producting species: Clostridium botulinum. Growth characteristics: *Cl. botulinum* displayed a predicted overall growth range, based on growth rate, of pH 5·1 to 6·8. Its growth rate above pH 5·9 was the fastest of the intoxicating group. (Fig. 1(c)). However, there is no predicted increase in numbers up to 7 days at pH values from 5·1 to 5·5 (Fig. 3(c)). *Cl. botulinum* also showed amongst the longest predicted lag phases of this group at between pH 5·1 and 6·2, which indicates its relative sensitivity to acid conditions. pH reduction would therefore seem to have potential for the control of this organism under the conditions established for the model (Fig. 2(c)). The effect of pH reduction is confirmed by the data presented in Fig. 3(c) although predicted numbers are higher than for the other organisms from pH 6·2 to 6·8.

Risk assessment zones: The definition of the safe phase is established at less than pH 5·5 in view of the low growth rates recorded in Fig. 1(c) and the lack of a predicted increase in numbers after 7 days (Fig. 3(c)). The caution zone is set between pH 5·6 and 5·7 because of the associated lag phases and reflects a potentially narrow margin of error. The critical and danger zones are set as indicated in Table 2 and it should be noted that the predictions for maximum growth rate yield a critical zone of 6·6–6·7 (i.e., less than the maximum pH value modelled of 6·8).

Staphylococcus aureus. Growth characteristics: *Staph. aureaus* showed the lowest predicted growth rates of the intoxicating types analysed although predictions of growth occurred at lower pH values than those predicted for *Cl. botulinum* (Fig. 1(c)). Lag phases predicted declined from a value of 33·4 days at pH 4·3 to 2·4 days at pH 6·1, which is comparable to *Cl. botulinum* in this group (Fig. 2(c)). No growth was predicted below pH 4·3 and at this pH, although a low growth rate was recorded (0·002 $\log_{10}$ increase in cell numbers h^{-1}), no increase in numbers was predicted after 7 days (Fig. 3(c)).

Risk assessment zones: A safe zone of less than pH 4·3 was set with a caution zone of

pH 4·4 to 5·0. A danger zone was established between pH 5·1 and 6·8 and, based on growth rates predicted, the critical zone lay between pH 6·3 and 6·7.

Thermal abuse

Thermal abuse was modelled as described in the **Materials and Methods** section, assuming an initial value of 10 cells with the same parameters for pH and NaCl, but with the temperature established at 22°C to reflect a typical ambient temperature. The exception was for *Y. enterocolitica* which was modelled using a temperature of 15°C owing to the limitations of the model. The results presented in Figs. 4(a–c) indicate that, with the exception of *Campylobacter* spp. (which was modelled using the survival model), all organisms increased in numbers with increasing time. *Cl. botulinum* showed the greatest predicted growth (Fig. 4(c)) followed by *Salmonella* spp. (Fig. 4(b)). *Campylobacter* spp. survived abuse, which is of significance (Fig. 4(b)). *Y. enterocolitica* exhibited least predicted growth but the lower temperature used certainly had an effect (Fig. 4(a)). However, even after 5 h of abuse there is a noticeable increase in all the organisms, which has serious implications for the safety of the product, especially for those pathogens having low infectious doses such as *E. coli* O157 being approximately less than 100 cells g^{-1} (ACMSF 1995, Eley 1996).

Such models are of considerable value in enabling predictions of effects of thermal abuse for various pathogens for differing times. This would be of value to the food industry in predicting the possible effects of out-of-process events such as the failure of storage refrigeration or problems in the cold distribution chain.

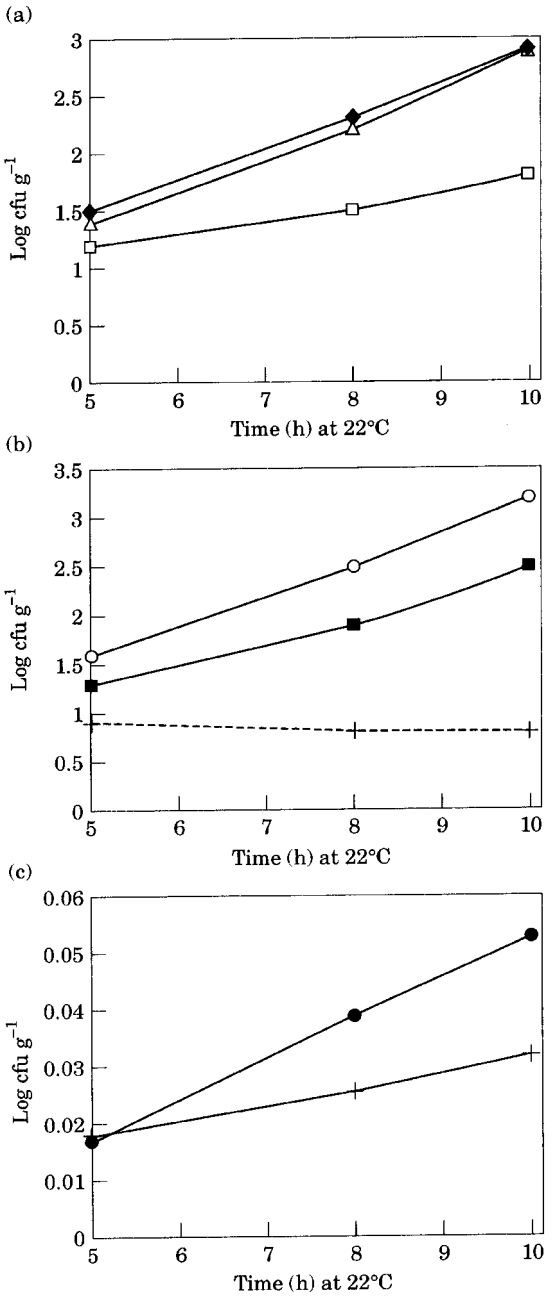

Figure 4. Predicted number of cells (log cfu g^{-1}) after 5, 8 and 10 h of thermal abuse at 22°C of: (a) *Aeromonas hydrophila* (△), *Yersinia enterocolitica* (□), and *Listeria monocytogenes* (♦); (b) *Salmonella* spp. (○), *Escherichia coli* O157:H7 (■), *Campylobacter* spp. (dotted line); and (c) *Clostridium botulinum* (●), *Staphylococcus aureus* (+).

Discussion

This study illustrates the possible use of FMM with a specified food system in several elements in the 'HACCP process'. General applications and uses of predictive microbiology can be found in previous review papers (Whiting and Buchanan 1994, Whiting 1995, McMeekin and Ross 1996). However, very

little has been reviewed on the *real* applications and uses of predictive microbiology instruments, particularly FMM, in risk assessment within HACCP plans and the benefits of using such models as a supporting tool in decision making.

Use of a semi-objective approach to identifying 'safety' zones to evaluate level of risk is initially of benefit since it may assist in identifying critical control points relating to product formulation and out-of-process events such as temperature abuse, tolerances for specific ingredients that effect a barrier to microbial growth, or extrinsic parameters such as processing temperature or storage temperatures. This approach may also be used to reformulate foods or modify storage temperatures to effect control. However, these zones are based only on a growth/no growth basis to prioritize hazards, which might not provide a complete understanding of risk; therefore, a more comprehensive assessment should include a complementary evaluation of the pathogens in terms of severity of disease, considering the infective dose together with their ability to survive rather than grow in the product. Thus pathogens which, according to the model, can potentially grow under the modelling conditions should then be evaluated in terms of severity of disease (including an evaluation of host parameters), infective dose and processing survival rate. For example, *E. coli* O157 and *L. monocytogenes* can cause severe diseases, which can be life-threatening in susceptible population groups, and, as both also have a low infective dose, survival represents the highest risk of the identified hazards. Using this assessment as a whole will enable the user to compare the pathogens not only between the safety zones but also within the safety zones when complementary assessments of severity of disease, infective dose and survival are carried out.

When conducting an HACCP plan, the first use of the FMM could be during the assessment of risks (Whiting 1995, Elliot 1996), in that the model can rapidly and objectively estimate which identified pathogens are of true significance in a given food product. Here, FMM works as a discriminatory tool among pathogens, ranking them in terms of importance.

The use of liver and other offal in pâté production, with a pH value close to neutrality as well as a mild pasteurization as the only potentially lethal step between the process line and the consumer, renders this product a high-risk food (Sprenger 1995). After hazard identification, the decision as to whether or not selected micro-organisms can survive, grow or die and therefore appear in the finished product can be estimated by use of FMM. According to the results presented above, the psychrotrophic pathogens (*Y. enterocolitica*, *A. hydrophila*, *L. monocytogenes*), because of their ability to grow at very low temperatures, would be ranked highest. *Salmonella* spp. showed comparatively low growth rates, for example, 0·025 $\log_{10}$ increase in cell numbers h^{-1} at 8°C, pH 6·8, 0·5% NaCl which is two or four (depending on pathogen) times slower than the pathogens mentioned above, and so it might be expected that *Salmonella* spp. would compete less well. However, continuous attention is required owing to the high contamination rate in farm animals. On the other hand, results revealed that *E. coli* O157 can grow at pH 6·8 in 0·5% NaCl at 8°C, doubling every 9 h, constituting a cause for concern. This is underlined by recent outbreaks in the UK (Wall et al. 1996) and also its low infective dose (ACMSF 1995, Eley 1996). *Cl. botulinum*, a potential threat in high-pH meat products also requires attention. *Staph. aureus*, although growing in the conditions modelled, would not seem to represent a major cause of concern because of the high levels of cells (10^6–10^7 cfu ml^{-1}) required for enterotoxin release, depending on substrate and for the type of enterotoxin (Jay 1996), and is not predicted in any of the models.

Following risk assessment, the HACCP team could consider adjusting one or more of the controlling factors (pH, a_w, temperature, %NaCl, preservatives) that affect microbial growth in order to guarantee the safety of a given product. The number of possible combinations of all those factors on the safety of products makes the use of challenge tests practically and economically unfeasible.

However, FMM allows rapid and quantitative estimations of the effects of sets of combinations of one or more of those factors on the growth of a wide range of foodborne pathogens. This assists in making more objective decisions on which product formulation is best to maintain a safety level at which the risk is kept at minimum. Unfortunately, that 'minimum' required is not always technically feasible and economically achievable with current technologies (Bernard and Scott 1995, WHO 1995); therefore, the formulation of the product should change in order to reach that minimum. FMM can therefore function as an iterative tool among all technically possible sets of combinations of the controlling factors which best ensure the safety level.

The present study evaluated the behaviour of the pre-selected pathogens vs acidic pH conditions. The initial hypothesis considered that lowering the pH could prevent the growth of pathogens in pâté and therefore can be used as a preservative means. The high pH and buffering power of pâté make it technologically unfeasible to reduce the pH below 5·0. The question of how safe the pâté will be at pH 5·0 can be readily answered with the use of the FMM. As summarized in Table 2 the reduction of the pH until 5·0 can only have a beneficial effect in decreasing the growth of *A. hydrophila*, *Cl. botulinum* and *Staph. aureus* and on survival of *C. jejuni*, but not for the rest of the pathogens for which the pH value of 5·0 is clearly within the limits of the danger zone. According to the model, *Salmonella* spp. *L. monocytogenes* and *Y. enterocolitica* can double cell numbers every 27, 16 and 7 h reaching 10^2, $1{\cdot}5 \times 10^2$ and 10^6 cells g^{-1}, respectively, in 7 days. Therefore, pH reduction alone is not an effective method against these pathogens, which is in agreement with experimental data (ICMSF 1996). The resistance of *E. coli* O157 to acidic conditions (Miller and Kaspar 1994, ACMSF 1995) allows this bacterium to have a doubling time of 14 h at 10°C and to approach 1000 cells in 7 days. Whilst this may be an overprediction of what could occur at 8°C, survival of *E. coli* O157 is expected to be enhanced when acidic conditions are combined with low temperature (4°C) (Miller and Kaspar 1994). Therefore the concern for that micro-organism is not only growth but also survival, which should be confirmed by challenge testing of *E. coli* O157 in pâté.

Predictive microbiology can be used as a system to set the criteria for each CCP, which means to establish target level(s) and tolerances that must be met to ensure the CCP is under control (Codex 1991). A CCP is under control if the hazard is eliminated or reduced to acceptable levels (Notermans et al. 1995). This is a potential third possible use of FMM in assisting HACCP teams as has been highlighted by Notermans and co-workers (1995). The question of which factors are controlling the CCP is easily resolved using FMM outcomes. Two circumstances need to be considered here: first, it is possible that the controlling factor is not found within the model's parameters, which means that additional challenge testing will be required; secondly, it is clear that in a food product the microbial load will not be represented by only one single species of micro-organism. Therefore it is necessary to evaluate whether or not the established preventive measure to control one given hazard is effective for others at the same CCP. The function of the FMM at that point is to work as an evaluative tool to verify which preventive measures (sets of factors) or which combination can eliminate or reduce all hazards at the same CCP to an acceptable level. When combinations of controlling factors cannot satisfy the desirable safe level or are unfeasible, the 'safety loop', proposed by Notermans et al. (1995), goes back to the beginning of the processing line where decisions on product or process modifications should be taken to guarantee that the acceptable level is satisfied.

Reducing the product's pH to 5·0 and maintaining a storage temperature of 8°C were not effective measures to prevent the growth of most pathogens in pâté. Some modifications are therefore needed to avoid the danger zones of the micro-organisms, namely, modify the product itself or one of its characteristics, modify the production process and/or modify the post-processing storage

conditions (Notermans et al. 1995). Clearly, the modification of choice in the present case should be the strict control of raw products in conjunction with a proper pasteurization process.

A fourth possible use of FMM operates once hazards have been identified and the product's formulation has been defined, in making it possible to link the hazards with the different steps of the process line, otherwise known as CCPs identification, principle 2 of the HACCP system (Codex 1991). FMM can locate CCPs where it is indicated that a certain level of a factor permits or suppresses microbial growth (Whiting 1995). In the present case study, it is possible to assert different CCPs throughout the product flow, assuming that good manufacture practices are well implemented in the company. The first CCP should be located in the incoming raw materials in order to obtain the highest hygienic class of meats from the suppliers, and to implement appropriate storage and stock rotation, which will have beneficial effects on the following steps of the process. The second CCP should be the product formulation, concerning pH, which will reduce the growth or survival of certain micro-organisms. The third CCP should be established in the heat treatment to ensure the destruction of all enteric bacteria including possible 'survivors' of the previous CCPs (i.e. *Salmonella*, *L. monocytogenes*, *Y. enterocolitica* and *E. coli* O157). Heating to pasteurization temperatures in pre-packed pouches followed by storage at 8°C will minimize the probability of cross-contamination and bacterial outgrowth, respectively. Finally, some dietary awareness should be specified on the label specially relating to *L. monocytogenes* and susceptible groups (Newton et al. 1993).

FMM may also be used in HACCP plans to assess the magnitude of process deviations (out-of-process events) when they occur (Whiting and Buchanan 1994, Whiting 1995). More objective and consistent decisions to establish corrective actions (principle 5 of HACCP) (Codex 1991) are therefore possible. Depending on the magnitude of the deviation, the stricter the corrective action needs to be or, in the worst-case scenario, decisions to rework, rapidly utilise, or scrap a food or ingredient can be taken without waiting for testing (Whiting and Buchanan 1994, Whiting 1995). The function of the FMM at that point is to work as a predictive tool to evaluate the magnitude of microbial growth or survival under an abusive (out-of-process) scenario. To illustrate the later assertion it was decided to predict the effect on the growth of the pathogens when the pâté was thermally abused (22°C), simulating a breakdown of the cool chain. Predictions showed a dramatic increase in the cell numbers at 8 and 10 h (Fig. 4(a–c)), indicating that the product will be unfit for human consumption and, once re-processed, it should be derived to animal food or other use.

Finally, the last (but not least) use of FMM in HACCP plans is to include all predictive data on the documentation and record keeping of the process, which is principle 6 of HACCP (Codex 1991). In the case of liability, the predictions can be used as an evidence of due diligence.

When working with FMM it is important to bear in mind that it is designed to be fail-safe, which means that models predict more growth than that naturally observed in food systems. Fail-safe predictions are clearly an advantage for those industries that want to be always fail-safe when assessing risks (Baird-Parker 1994). FMM, however, has been criticized in that it is a conservative model which could lead to more stringent critical limits being established that would be necessary to produce a safe product (Elliott 1996). This stringency may lead to overprocessing the product to limits that are technologically and/or economically unfeasible. However, it is the authors' opinion that within HACCP plans and risk assessment it is more important to be fail-safe that strictly precise. Moreover models are always subject to some kind of inaccuracy (Notermans et al. 1995, Whiting 1995, Roberts 1996b) which makes them unsuitable for accurate adjustment of processes (Notermans et al. 1995). In the FMM models, the confidence limits of the predictions can be measured using the root mean square error (RMSE), which measures the goodness of fit of the polynomial equation

to the data, and therefore can estimate the approximate error of the prediction of the growth rate (Sutherland et al. 1994). For *Staph. aureus*, *E. coli* O157:H7, *C. jejuni* and *L. monocytogenes* it is *c.*± 25−30%, and for *Bacillus cereus* the RMSE is somewhat higher, approaching 40% probably because it is a spore-forming organism (Sutherland, pers. comm.).

Consequently, predictions can provide very useful 'order of magnitude' or 'trends' of microbial growth (Notermans et al. 1995, Roberts 1996b) but which do not completely replace microbial testing nor the judgement of a trained and experienced microbiologist (Whiting 1995, Buchanan and Whiting 1996). Nonetheless, a good, quickly obtained trend is always a useful support for decisions when thousands of pounds are compromised.

In the present study, only pH was modified to assess the potential for pH reduction in the control of pathogens, and the predictions show that this approach permits a variable response in the pathogens identified. In this regard, FMM can be a useful instrument to support HACCP (during initial implementation and further maintenance of the system) but, owing to its limitations, cannot be a guarantee of product safety although it is useful to demonstrate whether a food or pro-

Table 3. Summary of the applications of Food MicroModel (FMM) predictive package in the maintenance and development of HACCP plans

HACCP Principles	Application
1. Conduct a hazard analysis	• Estimation of the risk: once micro-organisms have been identified the associated risk or probability of bacterial outgrowth under different conditions can be estimated: discriminatory application (Whiting 1995, Elliott 1996) • Determine the consequence of a microbial hazard in food. Growth, survival or death under different conditions can be estimated: discriminatory application (Whiting 1995, Elliott 1996) • Aid in the decision-making processes of risk assessment: FMM can be an objective supporting tool concerning microbial hazards (Elliott 1996)
2. Determine the Critical Control Points (CCPs) in the process	• Identification of critical steps in the process: a CCP can be established where the model indicates that a certain level of a factor permits or suppresses microbial growth: evaluative application (Whiting 1995)
3. Establish target levels (critical limits) and tolerances for preventive measures associated with each identified CCP	• Establishing ranges and combinations of process parameters as critical limits for CCPs: iterative use (Notermans et al. 1995, Elliott 1996)
4. Establish CCP monitoring requirements	• Describing processing parameters necessary to achieve an acceptable level of risk: iterative use (Notermans et al. 1995, Elliott 1996)
5. Establish corrective actions to be taken when monitoring indicates that a particular CCP is not under control	• Reformulation evaluations. The effects of several formulation variations can be estimated: evaluative application (Baker 1995, Notermans et al. 1995, Whiting 1995)
6. Establish procedures for verification that HACCP system is working correctly	• Objective evaluation of the consequences of lapses in process and storage control: evaluative application (Ross and McMecckin 1994, Whiting and Buchanan 1994, Whiting 1995, Buchanan and Whiting 1996)
7. Establish documentation concerning all procedures	• Inclusion of technical data concerning microbial growth at each CCP • Defend the safety in the case of liability (Baker 1995)

cedure might be unsafe. The main applications and uses of the FMM in the day-to-day work of the HACCP plan team leaders can be summarized as illustrated in Table 3.

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