Perfringens Predictor

For food microbiology

Developed at the
Institute of Food Research

User Manual

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Institute of Food Research
Norwich Research Park
Norwich
NR4 7UA, UK
Tel: +44(0)1603 255000
Fax: +44(0)1603 507723
Email: combase@ifr.ac.uk

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SECTION ONE: Introduction to Perfringens Predictor

1.1 What is predictive microbiology?

Predictive microbiology is the science of describing and predicting, by mathematical means, the response of microorganisms to their environment.

If sufficient responses are observed under varied combinations of environmental factors, then it is possible to predict how the organism will respond to any conditions within the environmental region where the observations were made. For example, by knowing how Clostridium perfringenswill grow at different temperatures it is possible to use mathematical equations to predict how it will grow during the cooling of meats.

1.2 Why might predictive microbiology and Perfringens Predictor be useful?

If you are involved in product development, then predictive microbiology will enable you to judge the effect of changing product formulations and/or processing techniques on the possible growth of pathogens or spoilage organisms. It facilitates risk assessment and can considerably reduce the amount of testing of intermediate or prototype products that is necessary. However, note that use of such predictions is not a substitute for the implementation of a HACCP procedure. Our other predictive microbiology programme (ComBase Predictor; modelling.combase.cc and food safety database browser.combase.cc are also very widely used as teaching aids.

Clostridium perfringens is an important cause of foodborne illness in the UK. It is commonly found in many foods including meat and poultry and is associated with food poisoning when cooked foods are subject to inadequate cooling. Slow cooling may allow germination of spores that have survived cooking processes, leading to rapid multiplication of the organism to an infectious dose. Once consumed, sporulation and production of the enterotoxin and associated illness can occur. Accurate prediction of the response of C. perfringensduring cooling processes will enable improved risk assessment and therefore an associated reduction in the extent of foodborne-illness related to this organism. Perfringens Predictor provides such a prediction.

1.3 What is Perfringens Predictor?

Perfringens Predictor is an extremely user-friendly computer-based tool developed to enable accurate prediction, through simulation, of the response of *C. perfringens* during varied cooling processes for foods which have been heat treated. Perfringens Predictor has been tested by ten potential users (e.g. Environmental Health Officers, food microbiologists from industry, appropriate international experts).

The software tool has recently been expanded to enable users to take account of the pH, NaCl (or water activity) and $NaNO_2$ concentrations of meat products when making predictions. This makes Perfringens Predictor a more useful tool, providing more accurate predictions in cured products and when the pH and NaCl concentration are not optimal. This web based application replaces the earlier Excel add-in version, and can be used to provide:

- Clear visual presentation of the predicted response of *C. perfringens*for a dynamic cooling profile
- Presentation of predicted response profile (X,Y) data alongside cooling profile (X,Y) data and the relevant chart

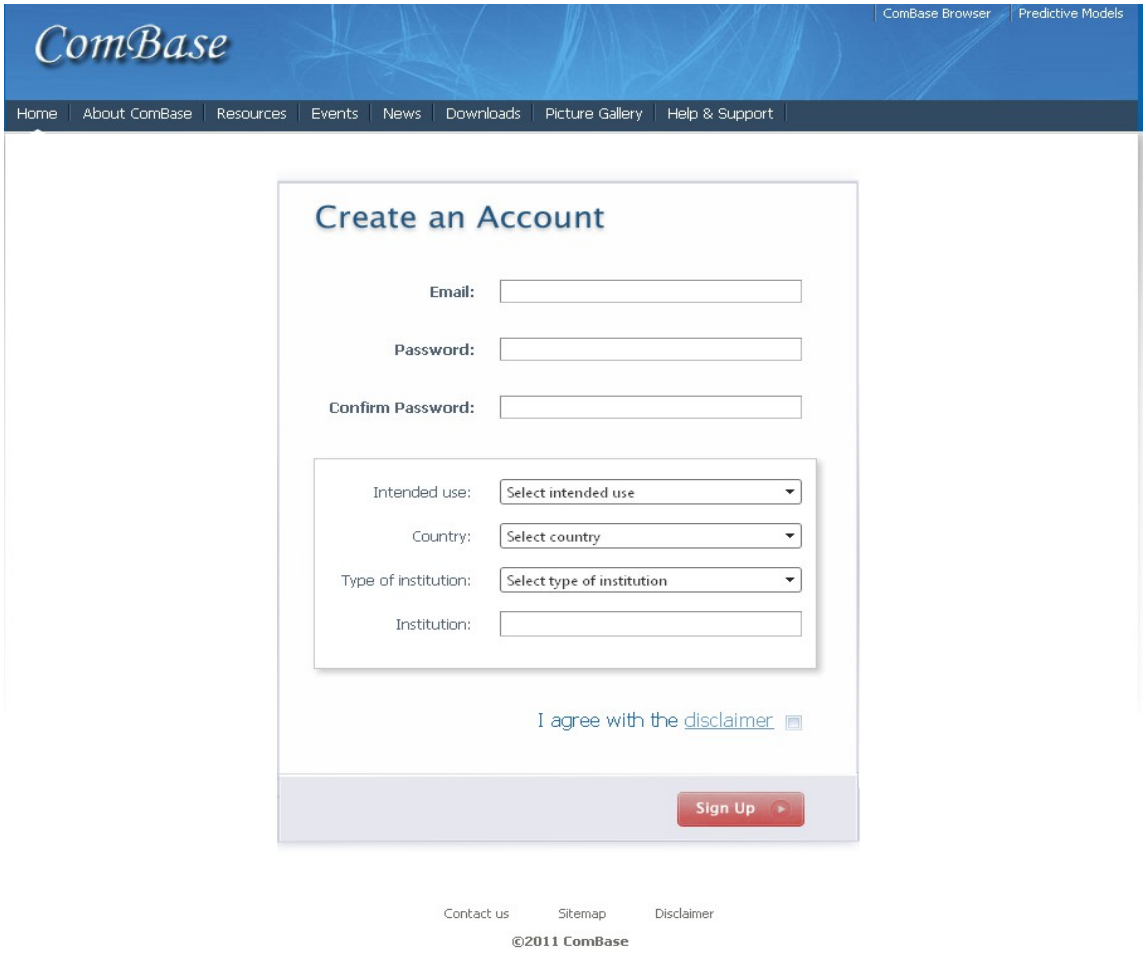
Details of how to use Perfringens Predictor are provided in section 2. Mathematical modelling and validation aspects of Perfringens Predictor are provided in the Appendix.

SECTION TWO: How to use Perfringens Predictor

2.1 Registration

It is necessary to register prior to using Perfringens Predictor. Your registration details will not be passed to third parties but, on request, we will add your contact details to our own mailing list so that we can provide you with occasional email alerts on important updates and major ComBase related events. To unsubscribe from the mailing list, log into your account and modify your account settings

To register, follow the instructions from the ComBase Predictive Models menu point in the www.combase.cc web page.

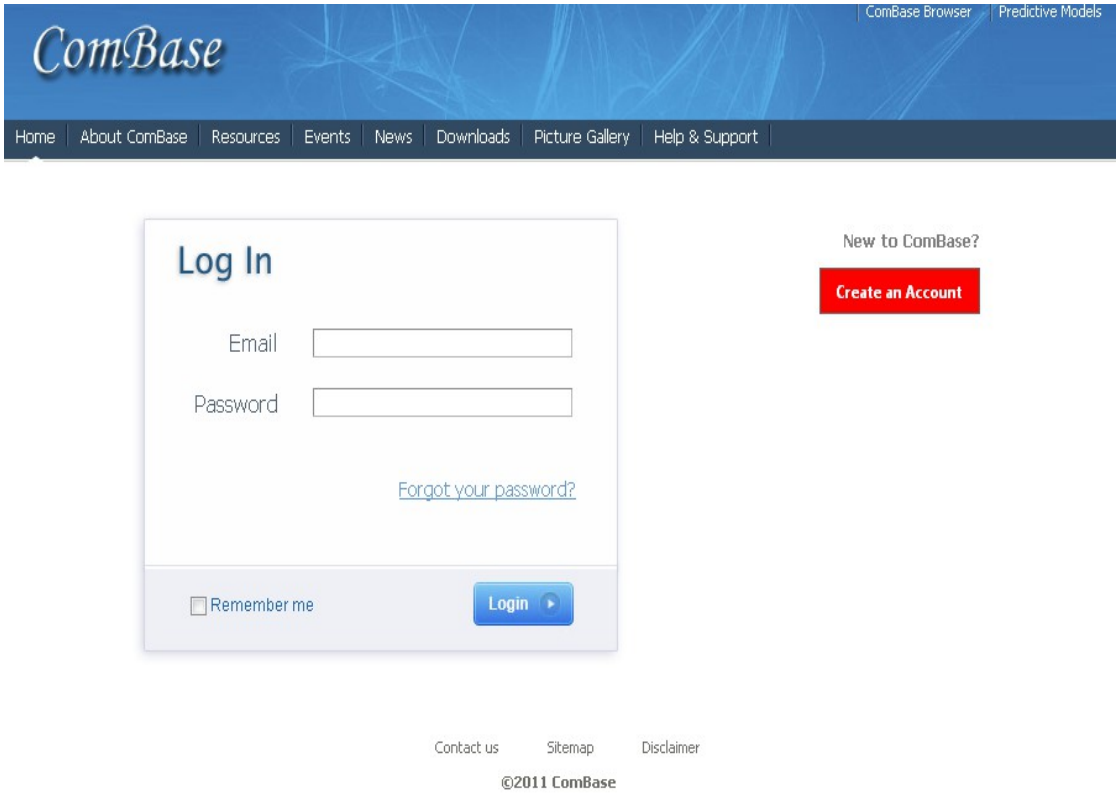


A message is automatically launched indicating whether the account has been successfully created or not. Note that only one account can be created for a given email address.

2.2. Login

Once your account has been created, you can login by typing your email address and password as shown in Figure 2.

Figure 2

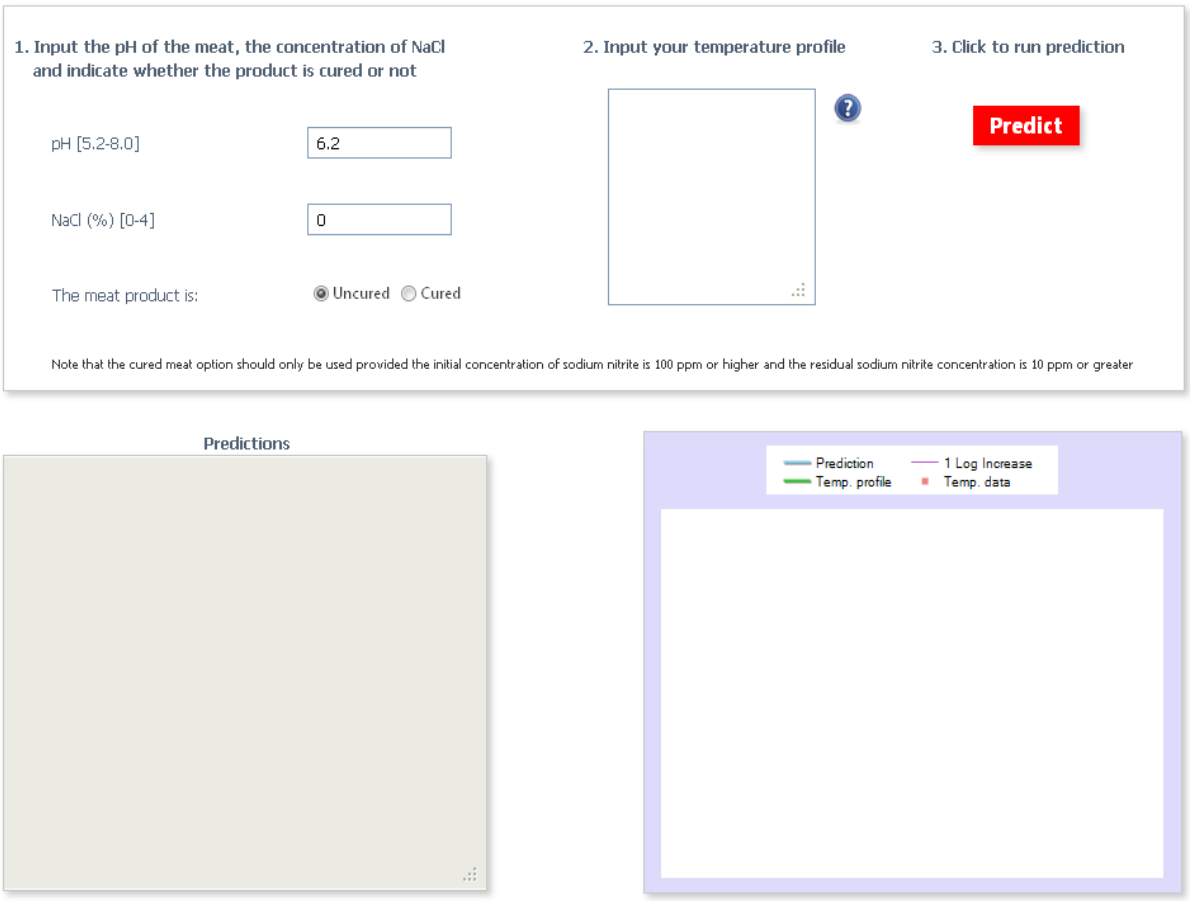


Login in brings up the default ComBase Predictive Tools page. Select 'Perfringens Predictor' to access Perfringens Predictor.

2.3 Using the programme

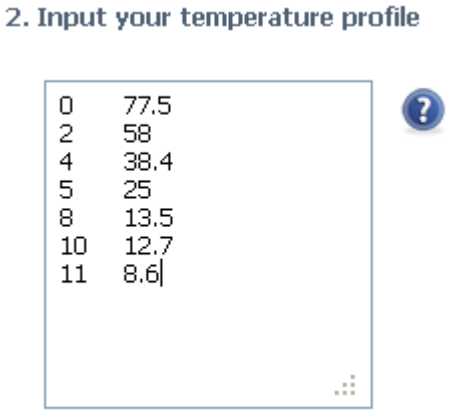
The Perfringens Predictor working screen now appears (Figure 3).

Figure 3



Time/temperature profile data should be inserted in the 'temperature profile' textbox in x, y columns of time, temperature as illustrated in Figure 4.

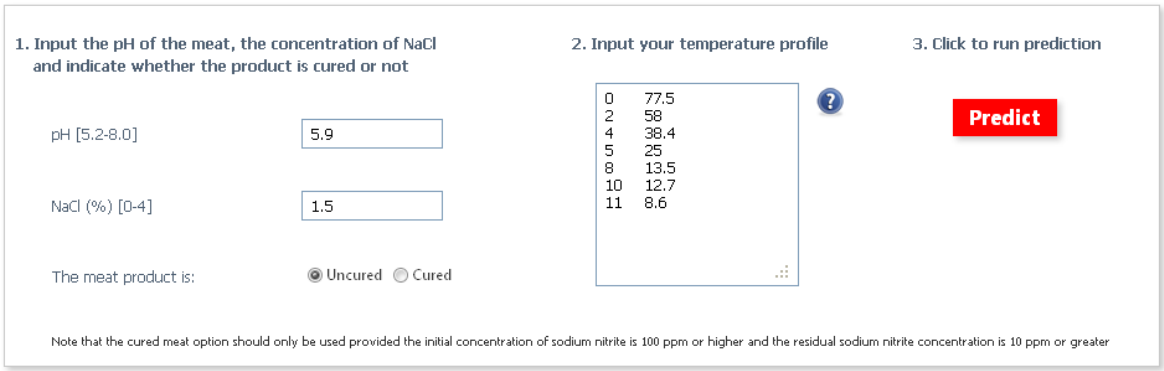
Figure 4

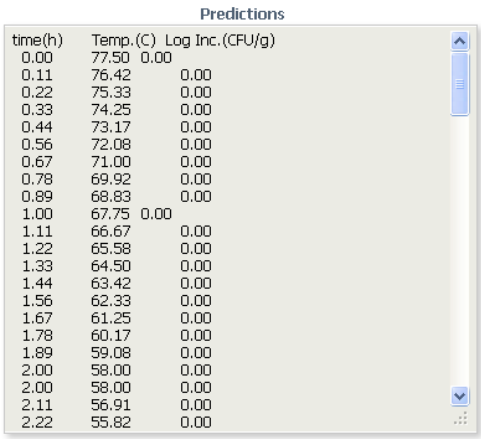


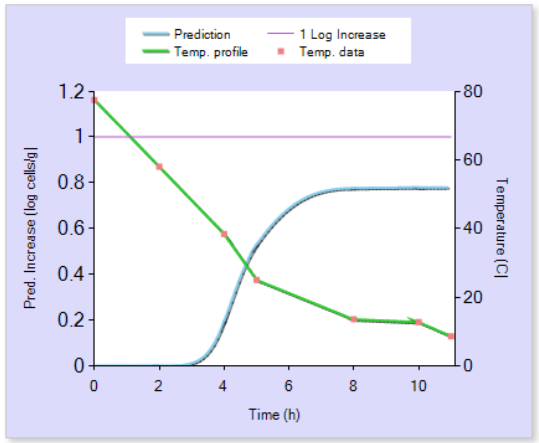
The profile can be typed directly into the 'changing temperature textbox' as follows: Time value, space, temperature value, return (repeat for each line). Only the numeric characters (0-9), space, and return are allowed in the textbox. Alternatively, the temperature data can be copied from another application (e.g. Excel spreadsheet, text file) and pasted directly into the text box. Note that the time unit is hours, and the temperature unit is degree centigrade (°C). The initial time must be zero hours, and the temperature must be between 0°C and 95°C. The final temperature must be less that 15°C. The temperatures should be those in the centre (warmest) part of the meat. A minimum of five time/temperature points are required.

Once the temperature profile has been entered in the textbox, input the pH value (5.2-8.0) and NaCl concentration (0-4%) and indicate if the meat is cured or uncured. The cured meat option should only be used if the initial concentration of $NaNO_2$ is 100 ppm or higher and the residual $NaNO_2$ concentration is 10 ppm or greater. Then click the 'Predict' button. The predicted response to the selected cooling condition will be displayed (Figure 5).

Figure 5







The temperature scale appears on the right hand Y-axis, and the input temperature data are displayed on the plot (Temp. data). The temperature profile is displayed on the plot as a green line with red marker points (Temp. profile). The left hand Y-axis indicates the scale for “Pred. increase” in concentration of C.perfringens , and appears as a solid line on the plot (Prediction). A horizontal purple line is also present indicating a predicted one log increase in concentration of C. perfringens. Generally, the slower the cooling of the meat, the more chance it has that growth from heat resistant spores of C. perfringens exceeds the one log increase.

Predicted values of the total log increase are also displayed in the ‘Predictions’ textbox.

If further predictions are required, then new values for pH, NaCl, $NaNO_2$ and time/temperature profile data should be entered as required, and the process described above repeated.

2.4 Interpretation of results

This program is intended for prediction of growth of *C. perfringens* during the cooling of meats that have been heat treated at 70°C-95°C. The meat must be cooled to less than 15°C for a prediction to be given.

The cooling of meat should be sufficiently rapid as to ensure that any growth from heat resistant spores of C. perfringens is minimal (e.g. no more than a one-log increase). This program predicts the extent of any increase in growth during cooling, and informs the user of whether cooling is sufficiently rapid. Perfringens Predictor has been validated for use with different meat types and preservative factors (see appendix A3 for further details).

APPENDIX: The model

This appendix provides supplementary information on the model. It is not necessary to read this section to use Perfringens Predictor.

A.1 The evolution of Perfringens Predictor

Since the early 1980s, with the advent of powerful PCs, food safety research has increasingly turned to quantitative, computational tools. The development of databases, hand in hand with mathematical and statistical processing, has become a key element in food safety. This principle was recognised by the UK Food Standards Agency (formerly the Ministry of Agriculture Fisheries and Food), who launched their Predictive Microbiology Programme at the end of the 1980s. Work funded under this Programme produced a large amount of growth and survival data for various food-borne organisms and formed the underlying database for the Food MicroModel (FMM) software. These data now constitute the core of the database, ComBase (www.combase.cc) which underlies the Combase Predictor which supersedes FMM and predicts the growth and survival of microorganisms in food.

Perfringens Predictor is a further development in this series of computer programmes for microbiological safety.
It is free of charge to the end user and it is based on the many of the same principles as Combase Predictor but has been specifically developed and validated to predict the response of *C. perfringens* in cooked meat during cooling, thereby enhancing risk assessment and therefore food safety with regard to *C. perfringens*

A.2 Development of the Perfringens Predictor model

Perfringens Predictor predictions are based on a model developed at the Institute of Food Research. The purpose of this model is to predict the lag phase and growth response of *C. perfringens* in meat during cooling.

Unlike some earlier models which predominantly used the Gompertz sigmoid function, Perfringens Predictor uses the Baranyi model (Baranyi and Roberts, 1994). Because of the dynamic background of this model, predictions can be generated for fluctuating temperature profiles. In Perfringens Predictor the dynamic model is solved numerically, by the fourth-order Runge–Kutta method. The model is defined by four parameters:

- y_0 the natural logarithm of the initial cell concentration.
- μ_{max} the maximum specific growth rate (dynamically changing with temperature)
- y_{max} the natural logarithm of the maximum cell concentration.
- α_0 describes the 'initial physiological state' of the spores (see Baranyi and Roberts, 1994)

Perfringens Predictorpredicts the net increase of *C. perfringens*in response to dynamic cooling conditions, considering a potential presence of *C. perfringens*spores at a concentration y_0. Predictions were performed, assuming a potential maximum 9 log increase of the bacterial concentration from the inoculum y_0.

The α_0 value is a dimensionless number between 0 and 1; if α_0=0, then there is no growth, and the lag time is infinite; if α_0=1, there is no lag, and growth will commence immediately. It has the role of an initial parameter, quantifying the history of the cells or spores. From that, the lag is a consequence, as the following formula in static conditions shows:

$$lag = -\frac{\ln(\alpha 0)}{\mu_{max}}$$

To solve the Baranyi model in fluctuating temperature, the temperature dependence of μ_{max}, the maximum specific growth rate must be modelled. Development of the growth rate model was carried out into two stages. 84 growth curves or growth rates were selected from the ComBase database (www.combase.cc) or produced from research carried out at the Institute of Food Research. Curves selected were obtained in meat or in culture medium:

- at static temperatures (ranging from to 15 to 52°C)
- for water activity values between 0.977 and 1
- for sodium nitrite concentrations between 0 and 150 ppm
- for pH values ranging from 5.2 to 8

For each growth curve, the maximum specific growth rate was estimated by fitting the model of Baranyi and Roberts (1994) to the experimental log counts.

Secondly, the following equation was fitted to the growth rates of *C. perfringens*calculated in the first step of the modelling:

$$\mu_{max} = \begin{cases} b(T - T_{min})^2 (1 - e^{c(T_{max} - T)})(aw - aw_{min})(2 - aw - aw_{min})(pH - pH_{min}) \\ \qquad (NO_{2\,max} - NO_2) \quad if \;\; pH < pH_s \\ b(T - T_{min})^2 (1 - e^{c(T_{max} - T)})(aw - aw_{min})(2 - aw - aw_{min})(pH_s - pH_{min}) \\ \qquad (NO_{2\,max} - NO_2) \quad if \;\; pH \geq pH_s \end{cases} \tag{1}$$

where μ_{max} is the maximum specific growth rate and T, pH, and NO_2, the temperature, pH and water acitivity respectively. b, c, T_{min} (theoretical minimum temperature for growth), T_{max} (maximum temperature for growth), pH_{min} (minimum pH for growth), aw_{min} (minimum aw for growth), NO_{2max} (maximum pH for growth) and pH_s (a threshold value above which μ_{max} does not change) are the parameters of the model. Before fitting, a square root transformation was performed to homogenize the variance of the growth rate. Estimated parameter values are provided in Table 1.

Table 1. Fitted parameters of the growth rate model

Parameter	Estimate	Standard error
b 0.03901		0.00866
T_{min} 12.20 0.87		
c 0.0947		0.0252
T_{max} 54.47 0.52		
pH_{min} 4.76 0.13		
pH_s 6.25 0.05		
aw_{min} 0.9755 0.0006		
NO_{2min} 191 15		

Lag is more difficult to model than the maximum specific growth rate as it depends not only on the current environmental conditions but also on the history (physiological state) of the spores. Spores that are damaged after a heat treatment may require more time to germinate and/or repair damage before growth commences. As previously mentioned, the Baranyi model describes the lag time through an α_0 value (a dimensionless number between 0 and 1) related to the physiological sate of the spores.

Perfringens Predictor has been developed to predict the growth of *C. perfringens* during cooling, after a heating process that is may be damaging for the spores. Therefore it is important to determine which α_0 values should be used to reflect the physiological state of *C. perfringens* after typical heating profiles which may be applied by the food industry.

Experiments carried out at the Institute of Food Research (maximum temperature in the range of 70-95°C) suggest that in cured products, a α_0 value of 0.001 should be used. However, in uncured products, an α_0 value of 0.001 should be used for NaCl concentrations less than 1% and a value of 0.01 for NaCl concentrations higher or equal to 1%. Perfingens Predictor automatically selects the appropriate value.

A.3 Validation of Perfringens Predictor

Perfringens Predictor has been validated for use with different meats. Examples from IFR of the observed kinetics of *C. perfringens* during meat cooling, and prediction obtained from Perfringens Predictor are illustrated in Figures 12 to 31. The meats used in these tests were sterile beef, pork, or turkey slurry, with the pH adjusted to values between 5.5 and 6.3, and NaCl concentration between 0.5 and 3.0%. $NaNO_2$ was added to some of the sterile pork slurries to give input concentrations between 10 and 60 ppm. Further examples from IFR of the observed kinetics of *C. perfringens* during meat cooling and prediction obtained from Perfringens Predictor are illustrated in Figures 32 to 36. The meats used in these tests were either sterile beef or sterile turkey slurry, with no added preservatives.

Examples of comparisons of observed kinetics of *C. perfringens* during meat cooling from the literature, and the prediction obtained from Perfringens Predictor based on the cooling curves are shown in Figures 37 to 49 and Table 2. Details of the actual meats are given in the Figure and Table legends.

Predictions from Perfringens Predictor are based on (i) the heat treatment being in the range of 70°C (for up to 6 hours) to 95°C (for up to 1.5 hours). If lower heat treatments are used, growth of *C. perfringens* might be faster than predicted by the model, while if higher heat treatments are used, growth of *C. perfringens* might be slower than predicted by Perfringens Predictor. The pH of the meat should be between 5.2 and 8.0, and NaCl concentration 0 to 4% (or water activity 0.977 to 1).

Key to Figures 12-31: Red dashed line represents the temperature profile; the solid blue line represents the predicted increase in viable count based on the cooling profile and pH/NaCl/$NaNO_2$; and the blue squares represent the viable counts. The meats used in these tests were sterile beef, pork, or turkey slurry. $NaNO_2$ (sodium nitrite) was added immediately prior to heating.

Figure 12. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.3, 0.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

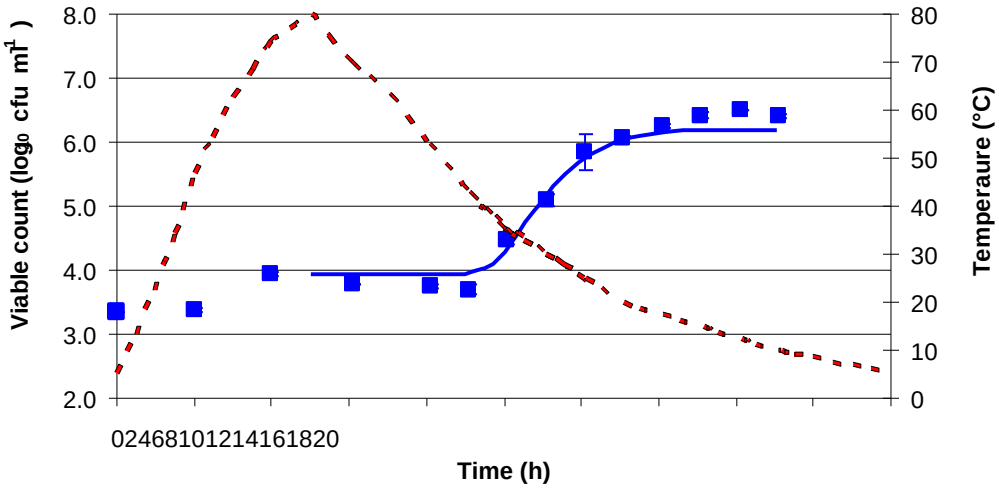


Figure 13. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 1.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

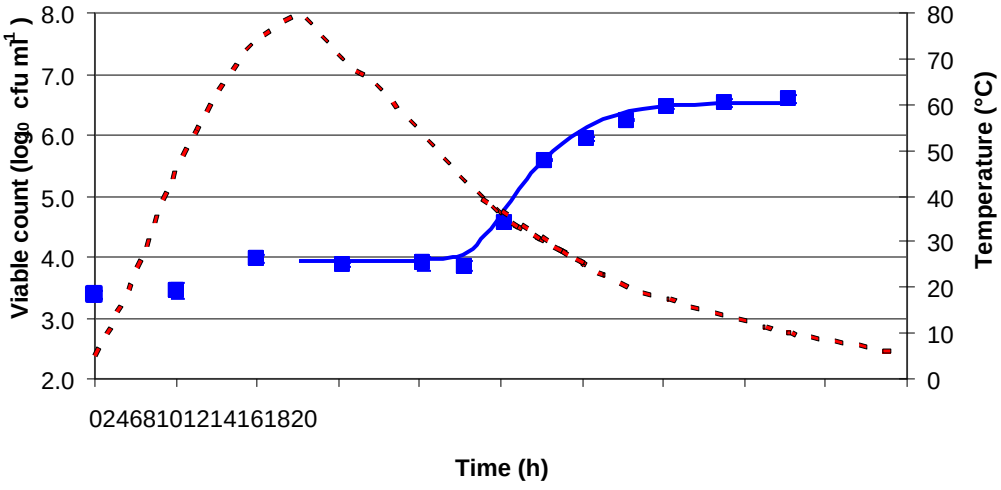


Figure 14. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 2.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

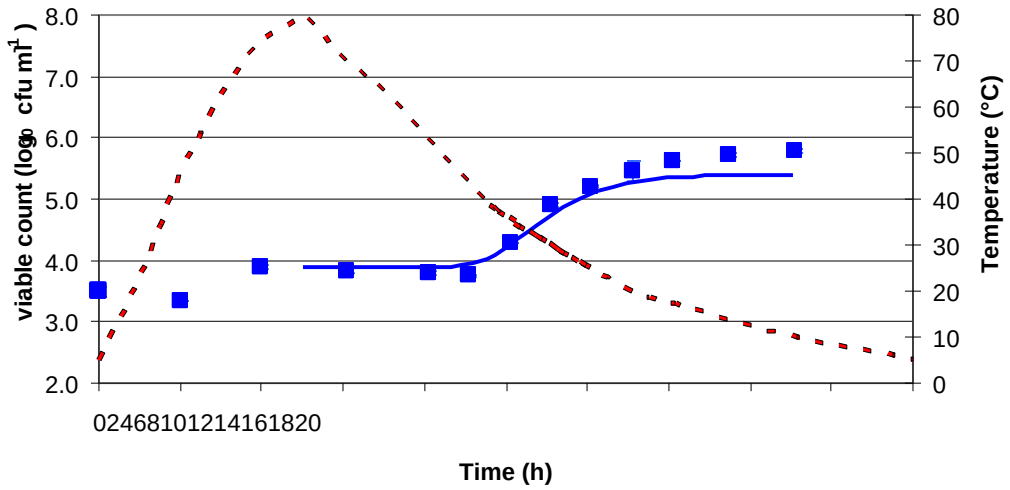


Figure 15. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 3.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

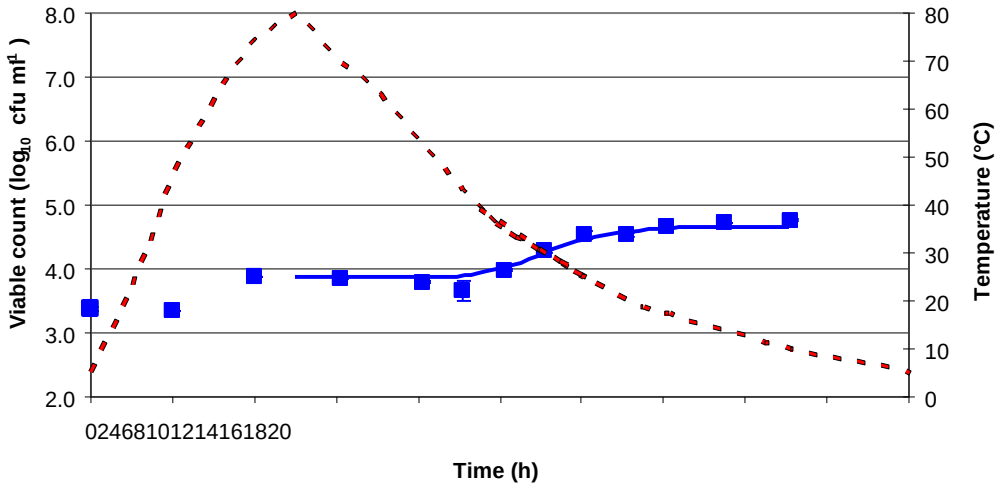


Figure 16. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 5.5, 0.5% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

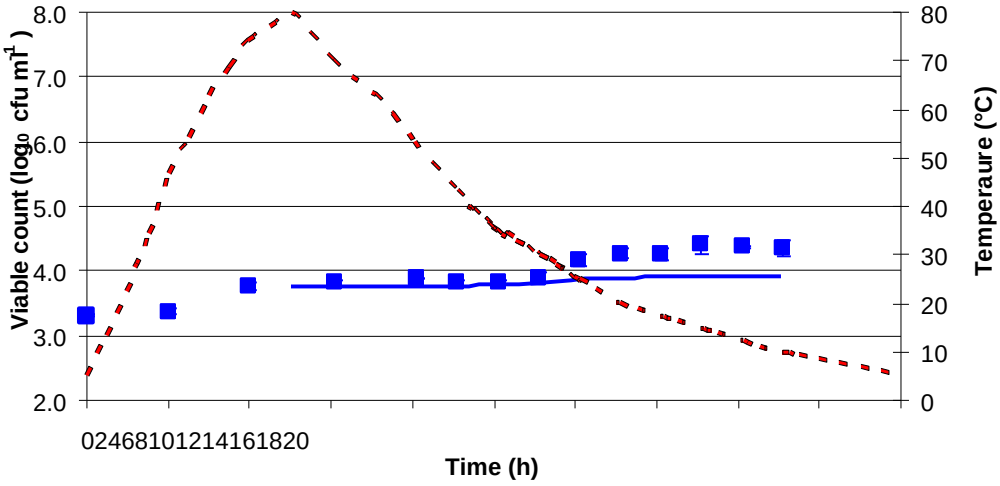


Figure 17. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 5.5, 3.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

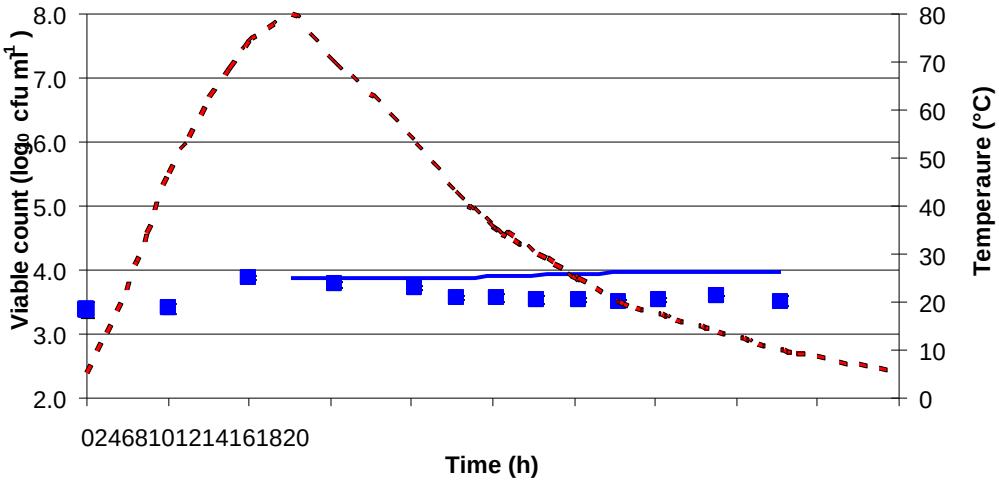


Figure 18. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 0.5% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

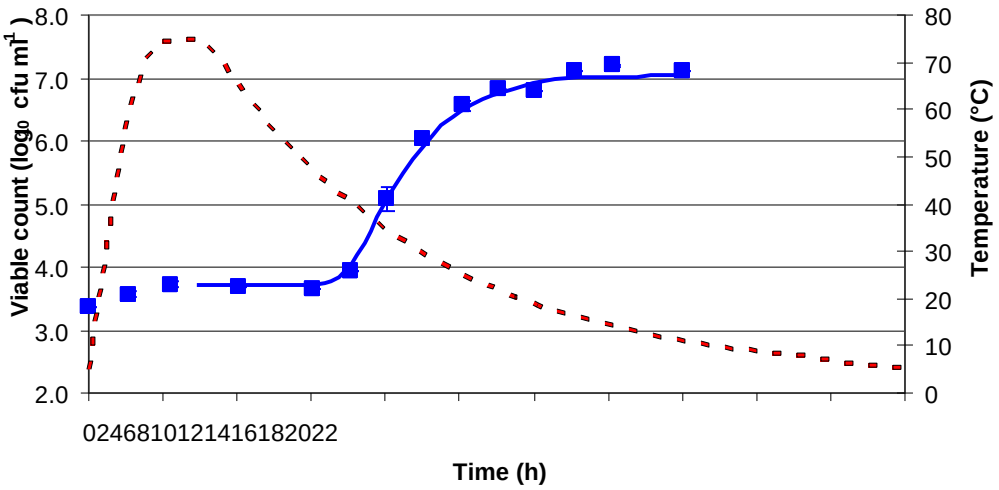


Figure 19. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 1.5% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

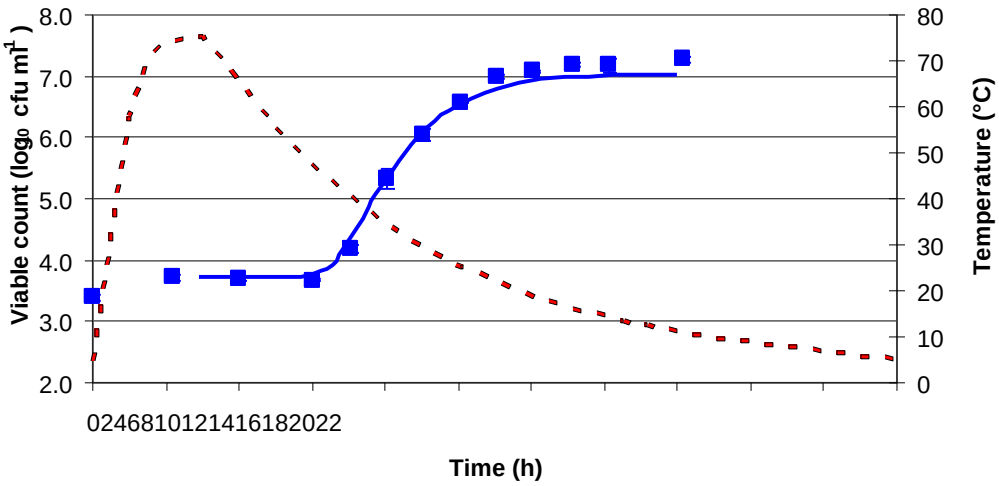


Figure 20. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 2.5% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

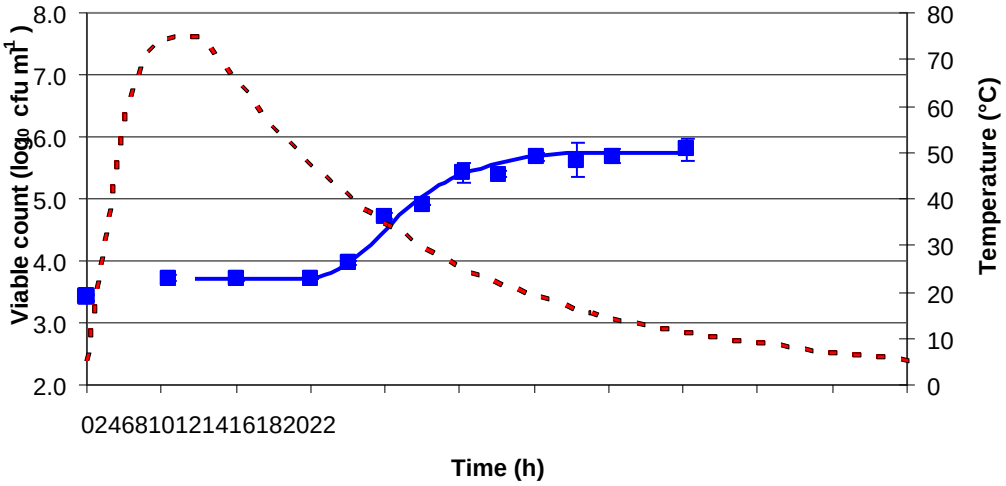


Figure 21. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 3.0% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

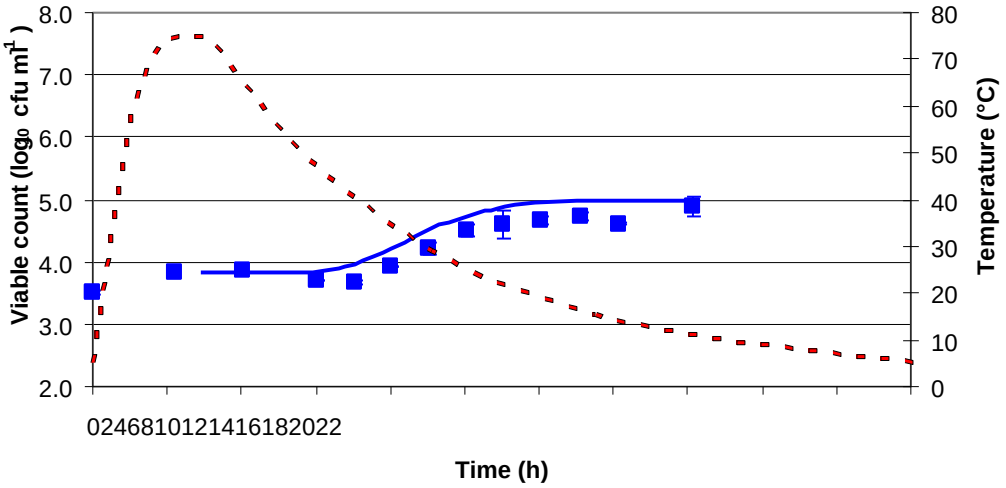


Figure 22. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 3.0% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

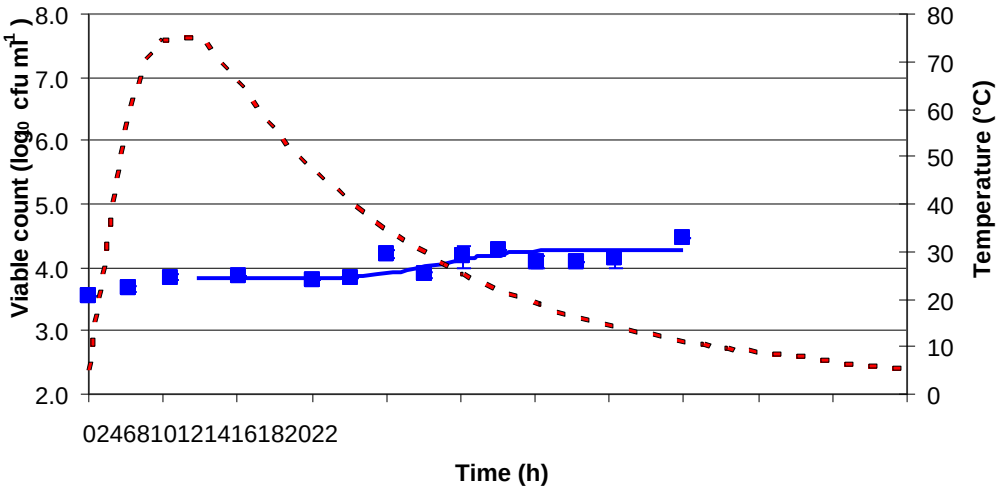


Figure 23. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in turkey slurry, pH 6.2, 3.0% NaCl after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

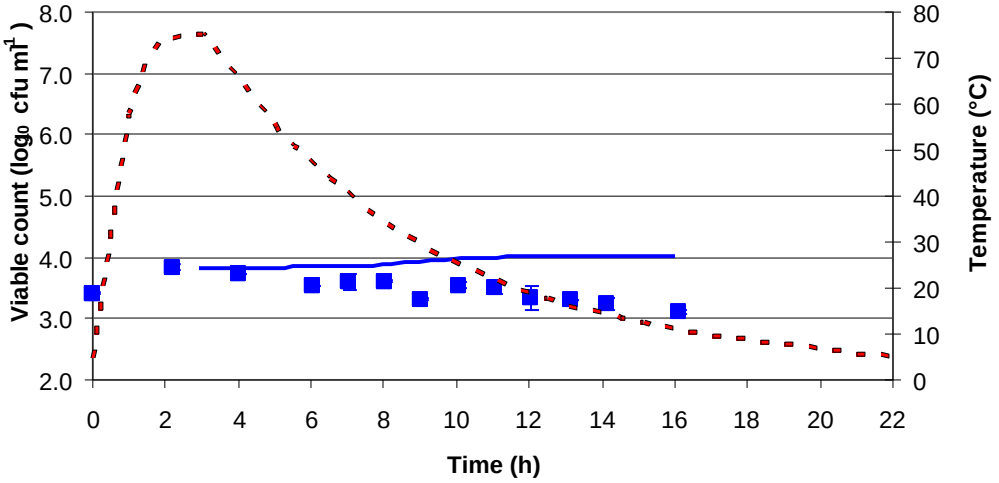


Figure 24. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 5.6, 1.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

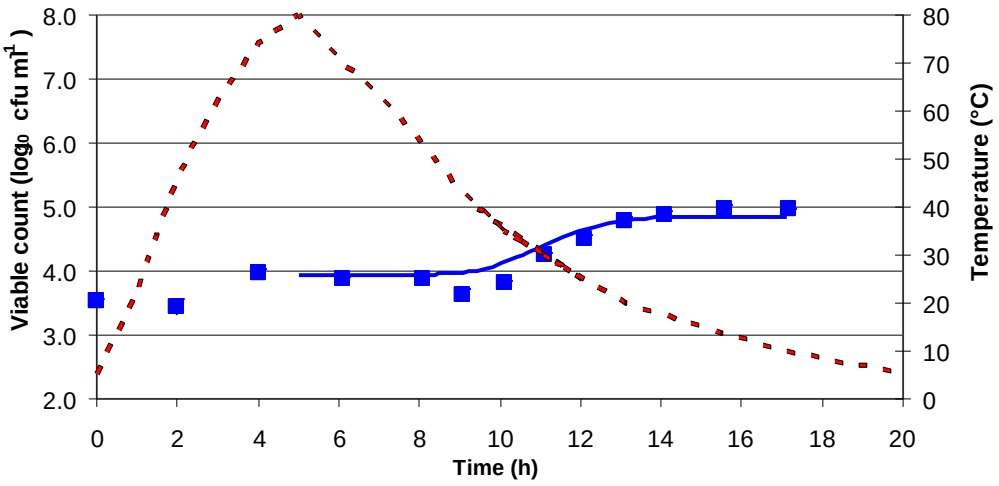


Figure 25. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 5.7, 2.0% NaCl after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

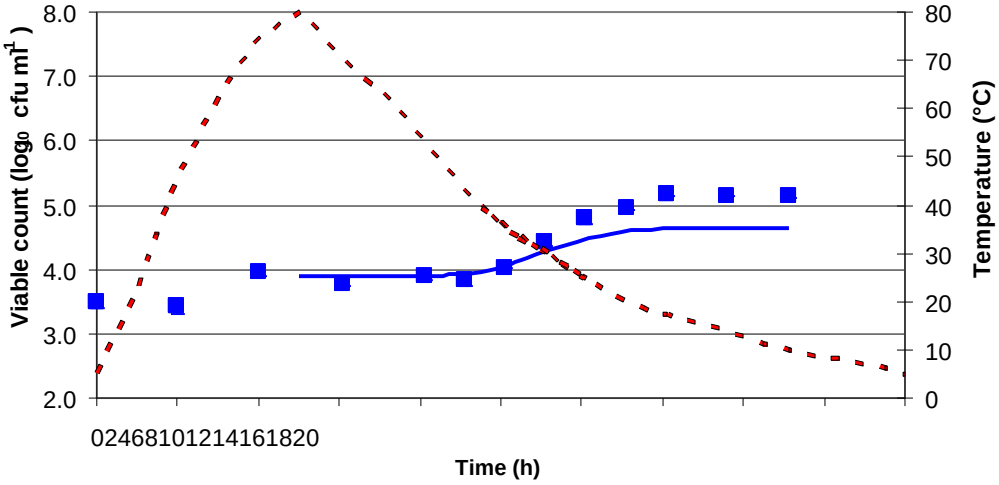


Figure 26. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.1, 1.5% NaCl, 10 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

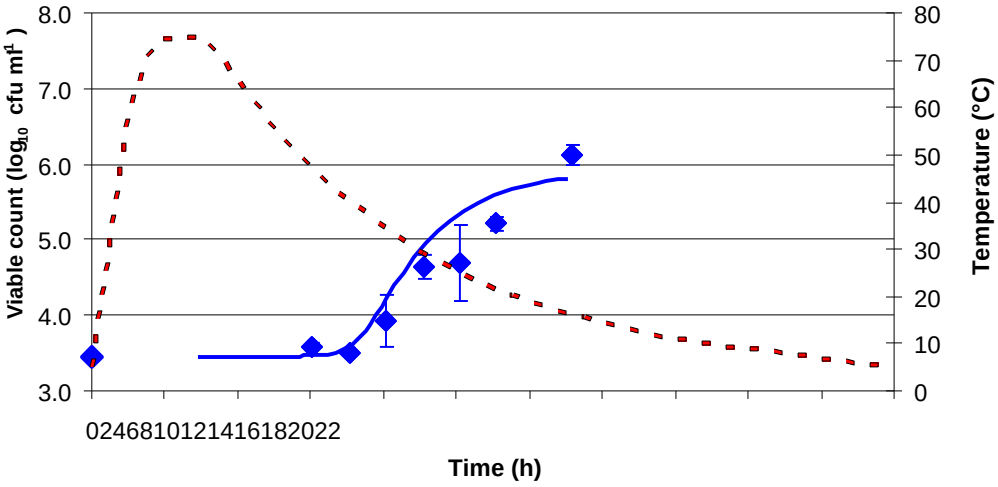


Figure 27. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.1, 1.5% NaCl, 15 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

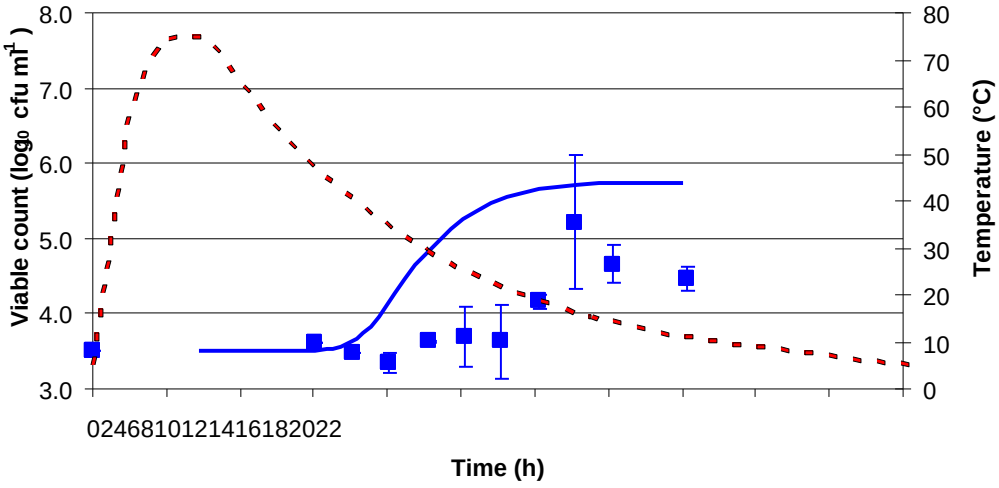


Figure 28. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.1, 1.5% NaCl, 60 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

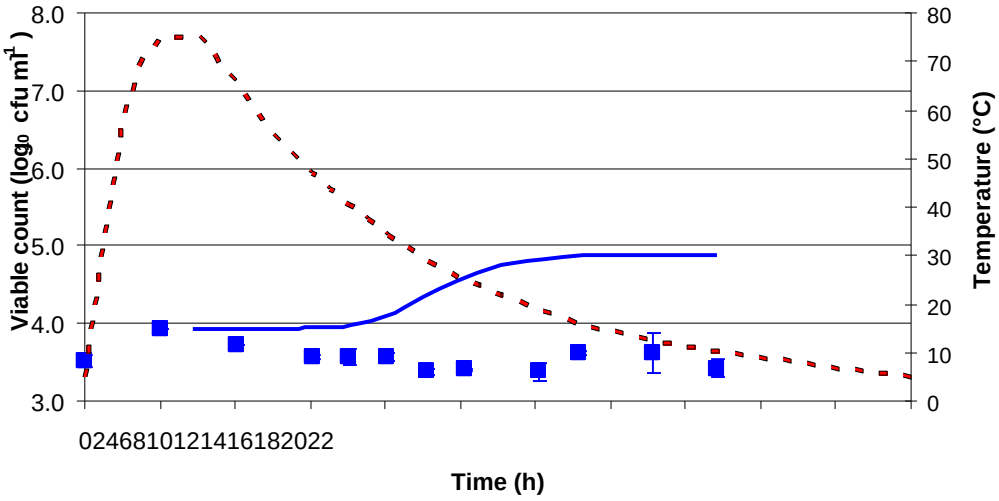


Figure 29. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.2, 2.5% NaCl, 10 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

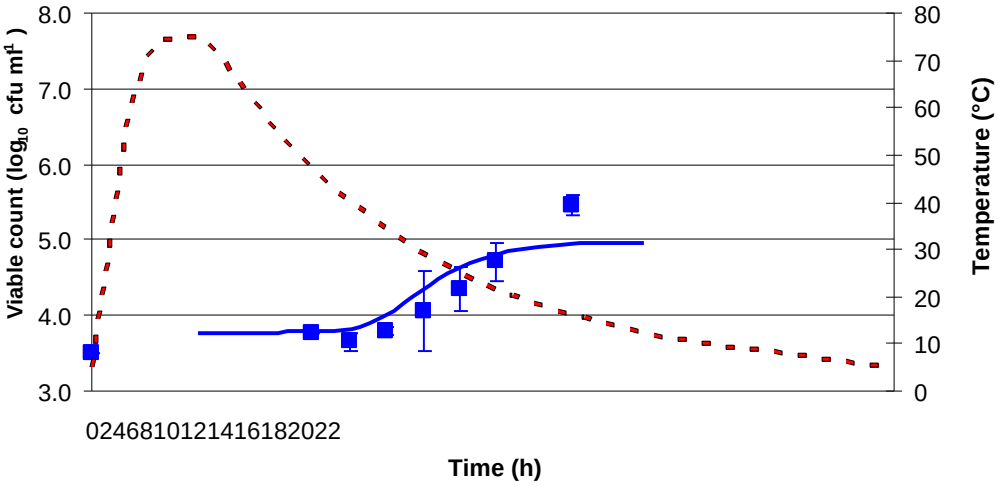


Figure 30. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.2, 2.5% NaCl, 15 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)

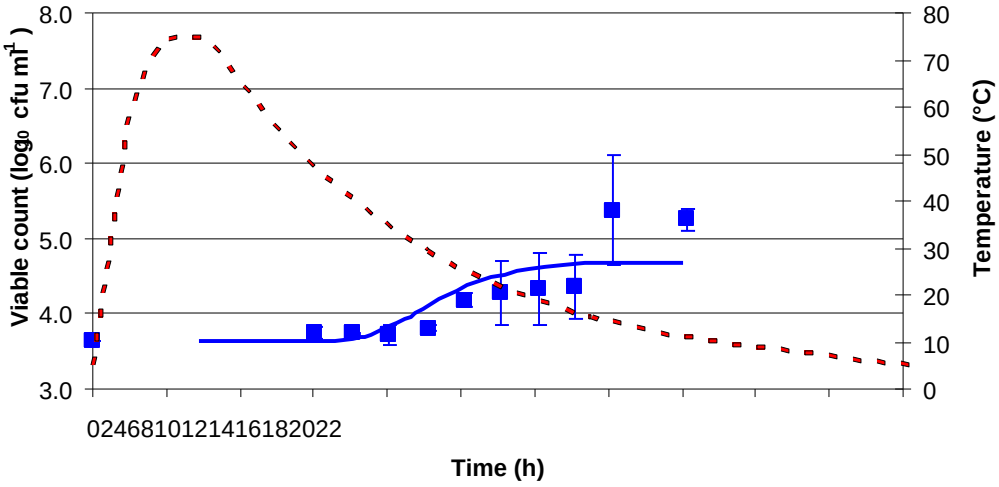
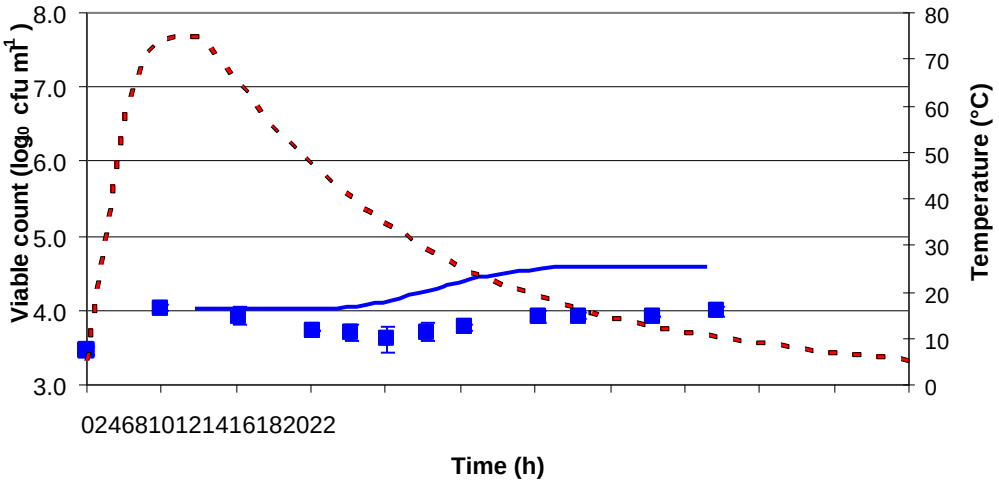


Figure 31. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in pork slurry, pH 6.2, 2.5% NaCl, 40 ppm $NaNO_2$ after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C)



Key to Figures 32-37: Red dashed lines represent dynamic cooling profiles; each of the solid green, blue or black profiles represent predictions for different starting concentrations of spores for respective heat treatments; and points represent experimental observations for those respective spore concentrations. The meats used in these tests were either sterile beef or sterile turkey, with no preservatives added.

Figure 32. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 2h heat-up from 5°C to 75°C, 1h at 75°C, followed by 19h exponential cooling to 5°C).

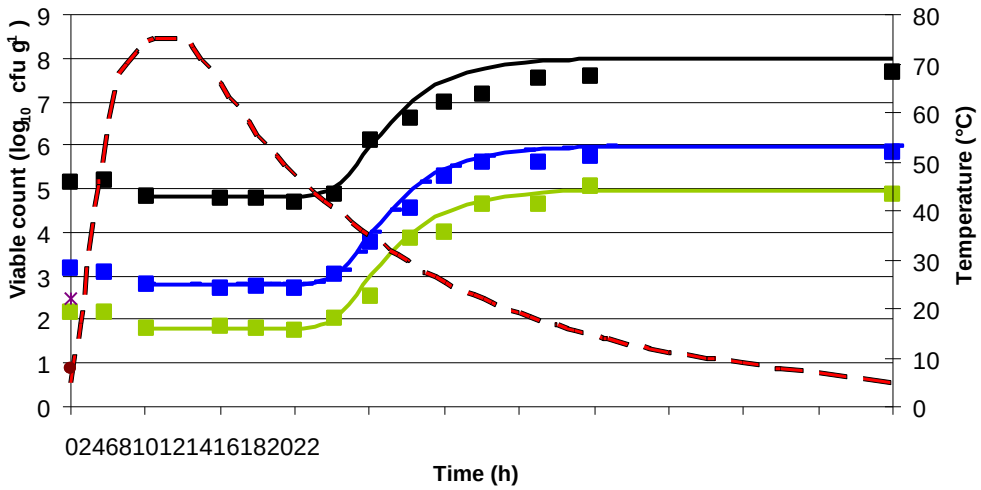


Figure 33. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 5h heat-up from 5°C to 80°C, followed by 15h exponential cooling to 5°C).

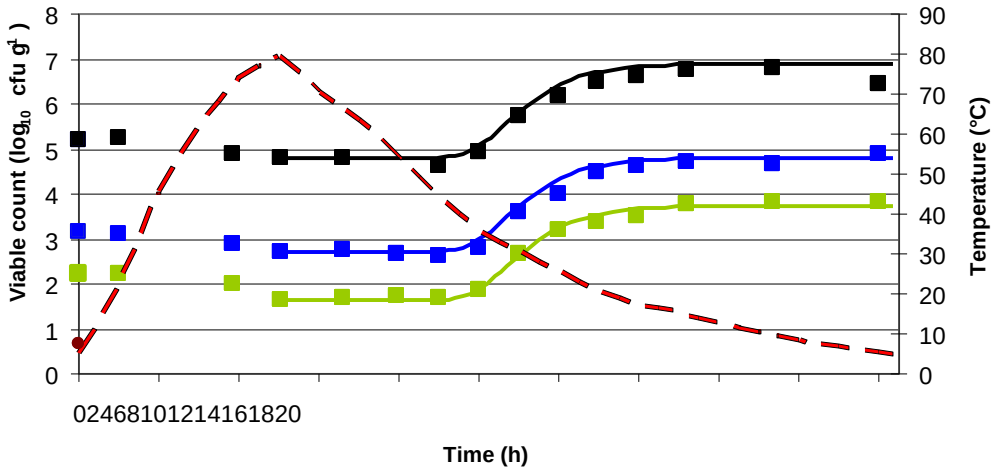


Figure 34. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 3h heat-up from 5°C to 75°C, followed by 7 h exponential cooling to 5°C).

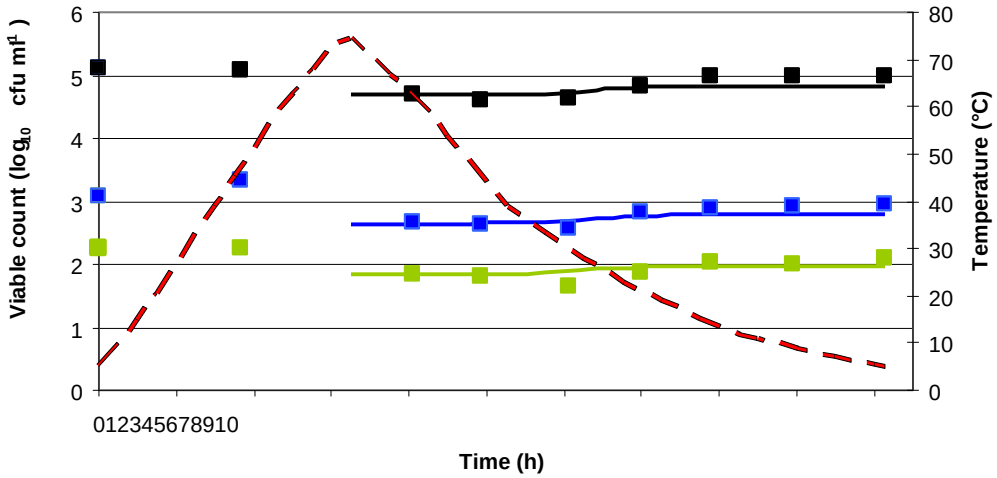


Figure 35. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 2h heat-up from 5°C to 95°C, 95°C/1h followed by 19h exponential cooling to 5°C).

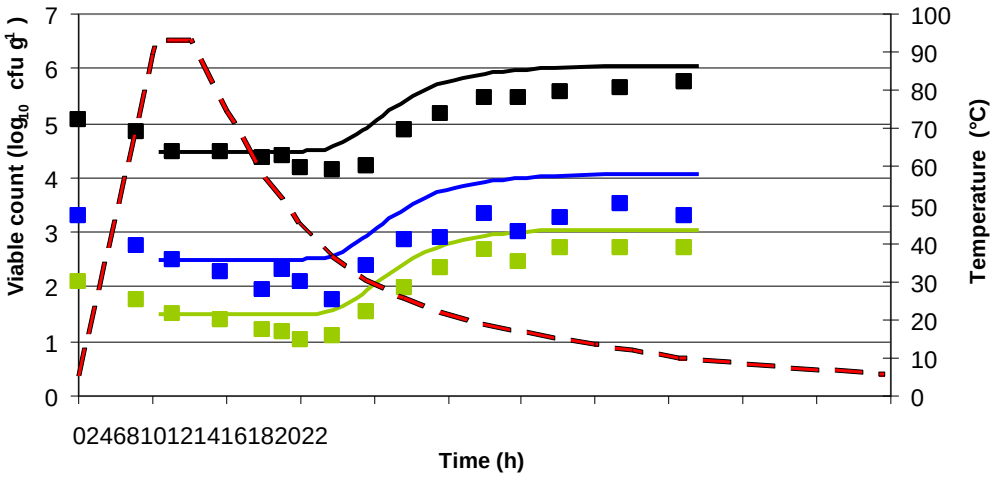
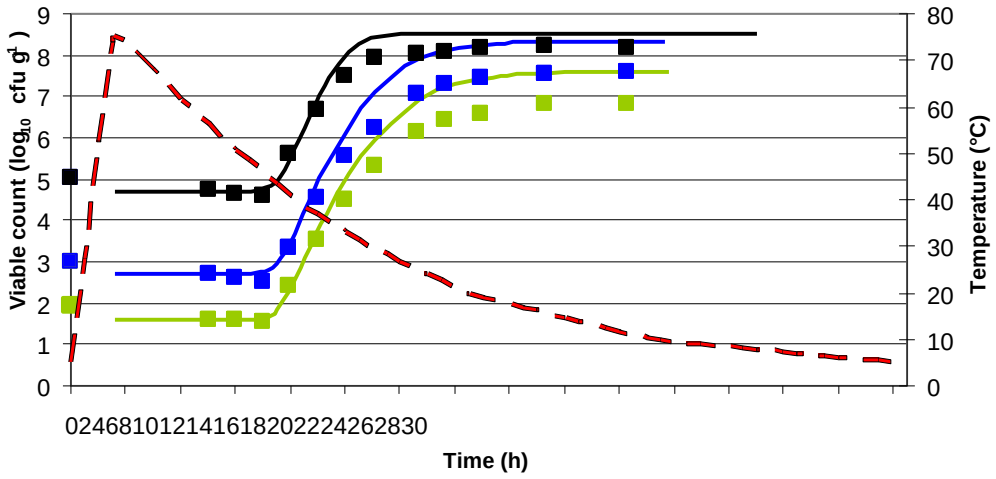


Figure 36. Comparison of output from Perfringens Predictor with observations made at IFR. (Kinetics of *C. perfringens* in beef slurry, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), after 2h heat-up from 5°C to 75°C followed by 28h exponential cooling to 5°C).



Figures 37 to 49 show examples of comparisons of observed kinetics of *C. perfringens*during meat cooling from the literature, and prediction obtained by Perfringens Predictor. Details of the actual meats are given in the figure legends.

Key to Figures 37-49: Red dashed lines represent dynamic cooling profiles, the solid black profiles represents the prediction from Perfringens Predictor, points represent experimental observations reported by the original authors.

Figure 37. Comparison of output from Perfringens Predictor with observations of Smith et al. [2004]. (The experimental system was ground beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation) with cooling from 54.4°C to 4.4°C in 21 hours).

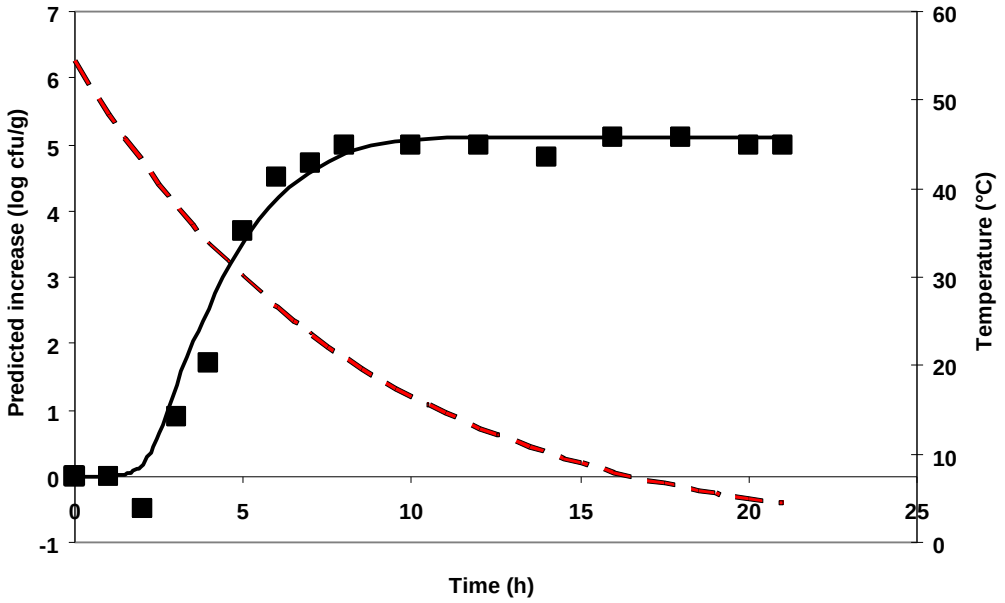


Figure 38. Comparison of output from Perfringens Predictor with observations of Smith and Schaffner [2004]. (The experimental system was broth, (conditions to be added) and the spores were heated at 75°C for 20 min (for activation)).

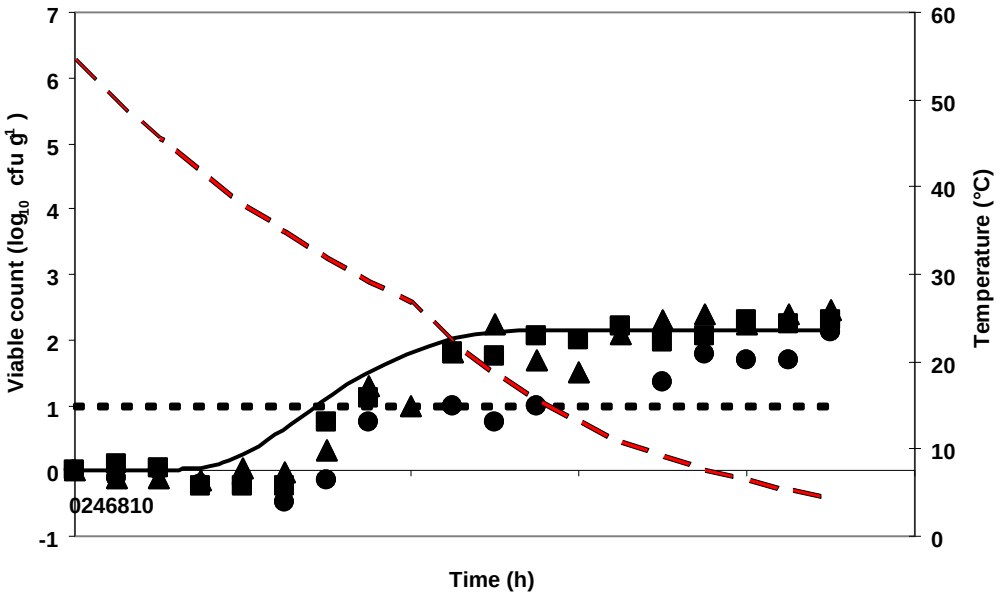


Figure 39. Comparison of output from Perfringens Predictor with observations of Blankenship et al [1988]. The experimental system was cooked chilli, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

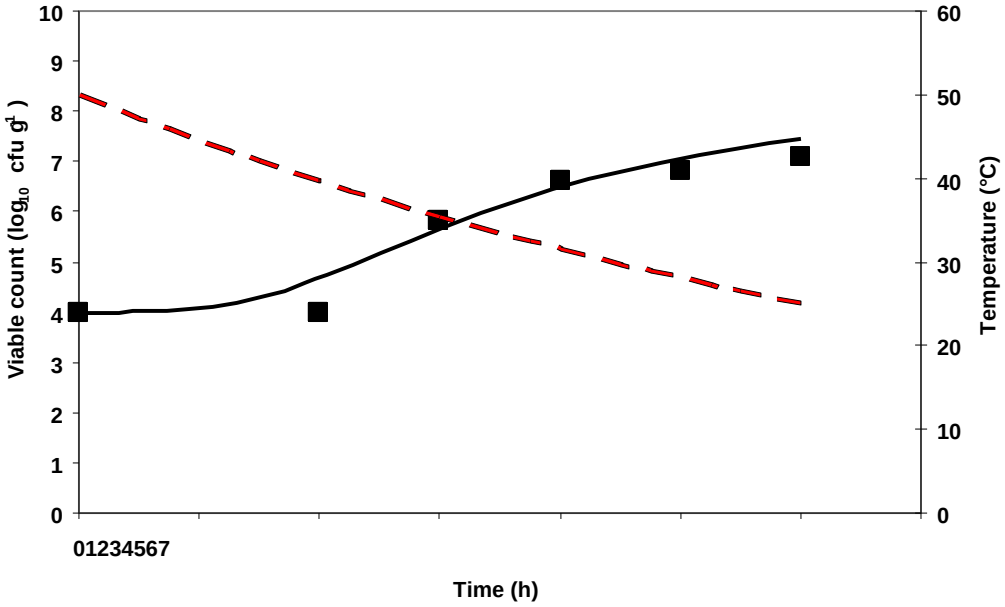


Figure 40. Comparison of output from Perfringens Predictor with observations of Shigehisa et al. [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

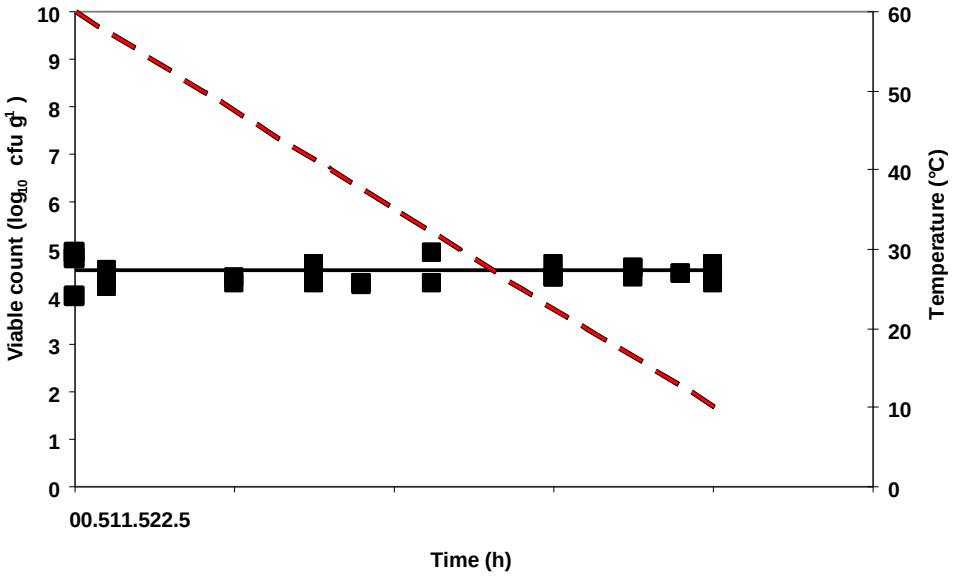


Figure 41. Comparison of output from Perfringens Predictor with observations of Shigehisa et al. [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

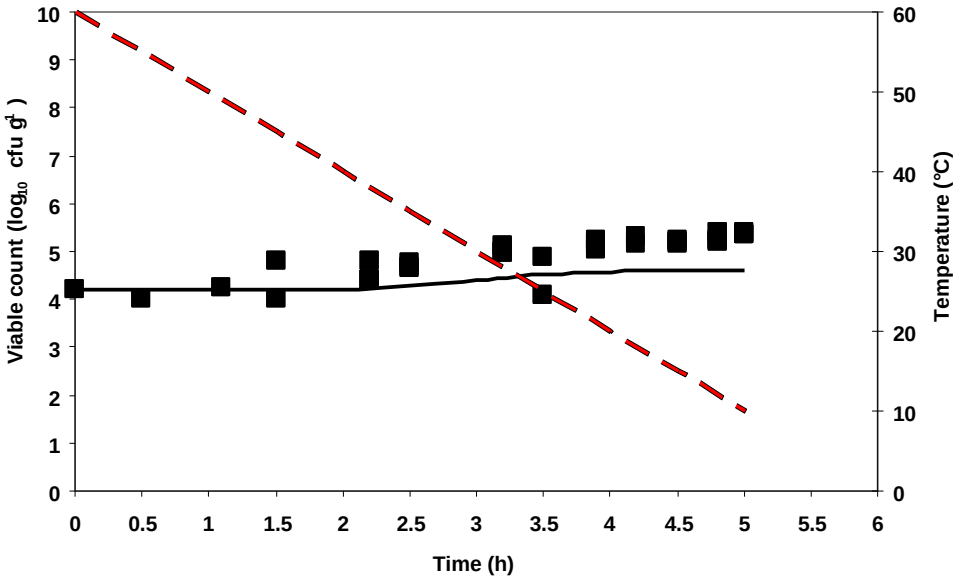


Figure 42. Comparison of output from Perfringens Predictor with observations of Shigehisa et al. [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

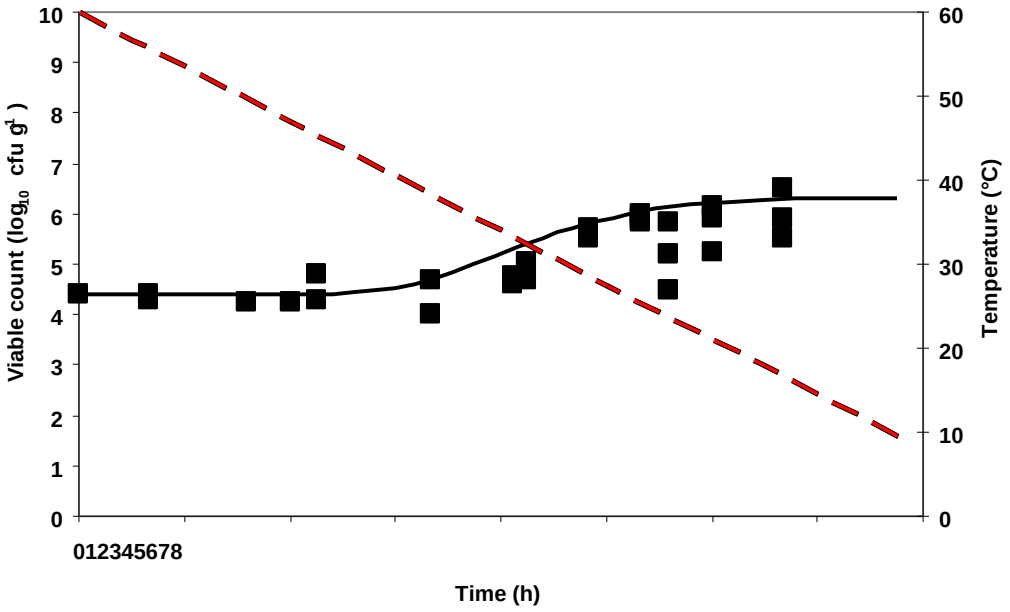


Figure 43. Comparison of output from Perfringens Predictor with observations of Shigehisa et al. [1985]. (The experimental system was heat treated mince turkey, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 80°C for 15 min (for activation)).

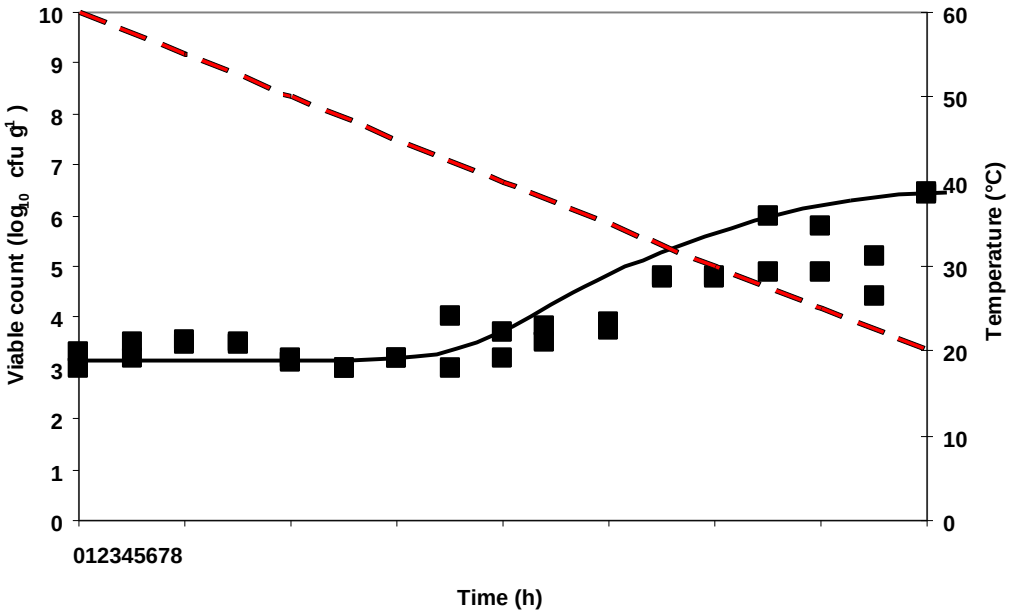


Figure 44. Comparison of output from Perfringens Predictor with observations of Sanchez et al. [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

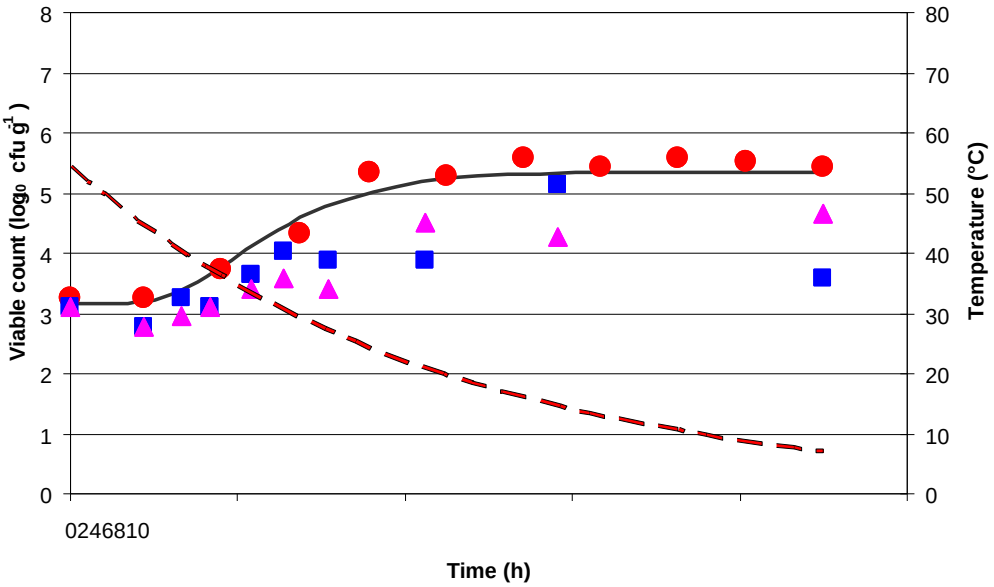


Figure 45. Comparison of output from Perfringens Predictor with observations of Sanchez et al. [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

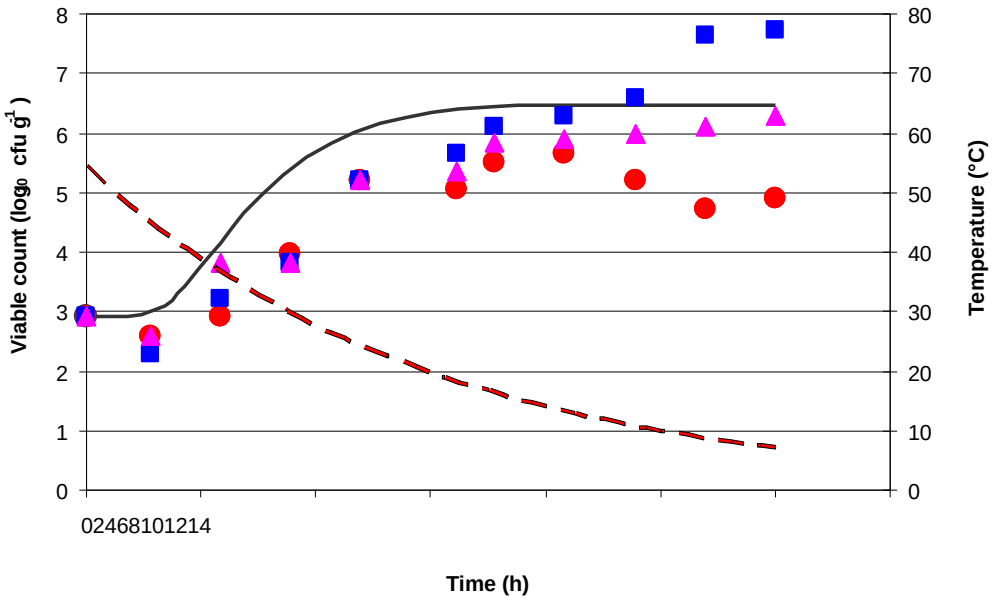


Figure 46. Comparison of output from Perfringens Predictor with observations of Sanchez et al. [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

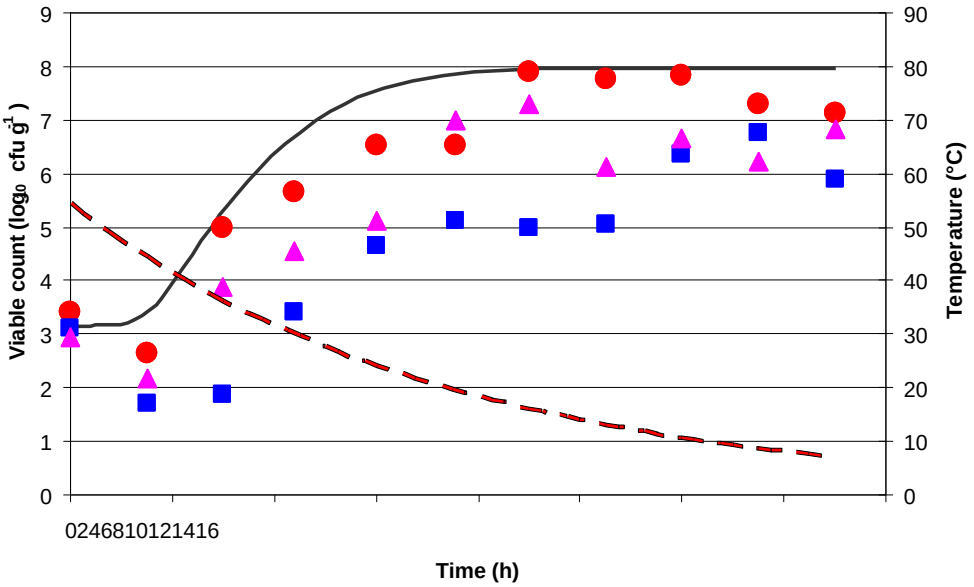


Figure 47. Comparison of output from Perfringens Predictor with observations of Sanchez et al. [2005]. (The experimental system was heat treated mince beef, pH 6.2, 0.5% NaCl, $NaNO_2$ (0 ppm), and the spores were heated at 75°C for 20 min (for activation)).

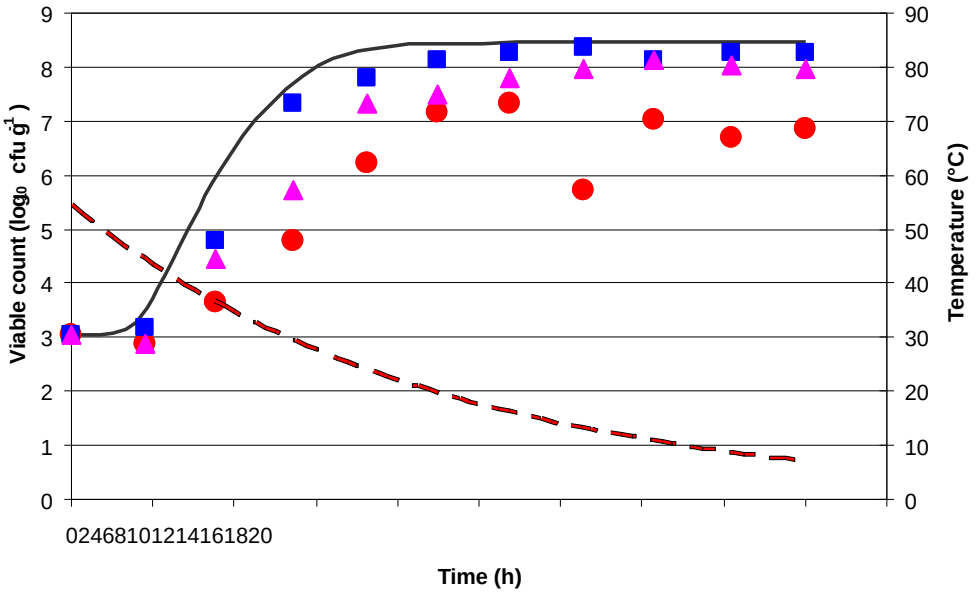


Figure 48. Comparison of output from Perfringens Predictor with observations of Amezquita et al. [2005]. The experimental system was cured ham pH 6.1, containing 50 ppm $NaNO_2$ (estimated to be the concentration at the start of cooling) and 1.25% NaCl. The spores were heated at 75°C for 20 min (for activation).

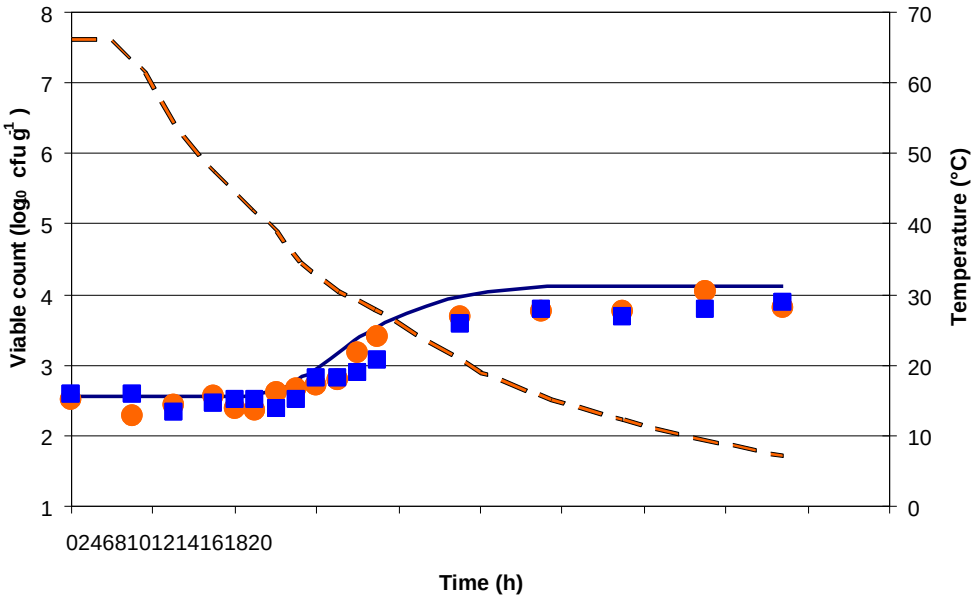


Figure 49. Comparison of output from Perfringens Predictor with observations of Amezquita et al. [2005]. The experimental system was cured ham pH 6.1, containing 50 ppm $NaNO_2$ (estimated to be the concentration at the start of cooling) and 1.25% NaCl. The spores were heated at 75°C for 20 min (for activation).

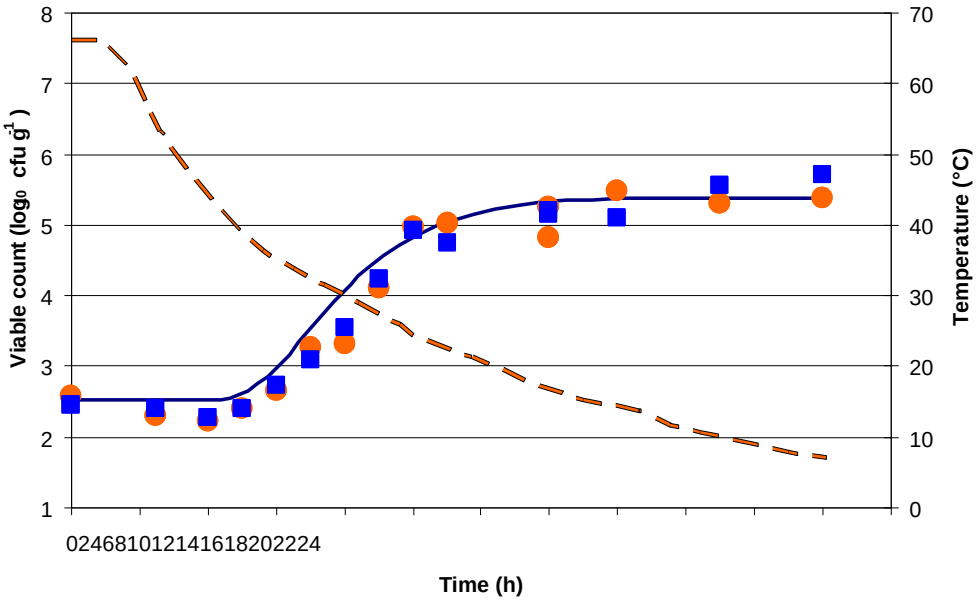


Table 2. Comparison of output from Perfringens Predictor with observations of Thippareddi et al. [2003]. The experimental system was roast beef (pH 5.6) or injected pork (pH 6.0), 0.5% NaCl, $NaNO_2$ (0 ppm). The spores were heated at 75°C for 20 min (for activation).

Meat	Cooling time	Observed log increase		Predicted log increase
		Mean	Maximum	
Roast beef	18 h	1.5	3.5	2.0
	21 h	5.3	5.4	3.0
Injected pork	18 h	3.7	4.5	3.4
	21 h	4.4	5.1	4.8

A.4 References:

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Shigehisa T, Nakagami T, and Taji S. (1985). Influence of heating and cooling rates on spore germination and growth in Clostridium perfringens in media and in roast beef. Japan. J. Vet. Sci. **47** 259–267.

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Thippareddi, H., Juneja, V.K., Phebus, R.K., Marsden, J. L. and Kastener. (2003). Control of *Clostridium perfringens* germination by buffered sodium citrate during chilling of roast beef and injected pork. Journal of Food Protection **66** 373-381.