Application of Modelling Techniques in the Food Industry: Determination of Shelf-Life for Chilled Foods

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Abstract

Microbiological modelling techniques (predictive microbiology, the Bayesian Markov Chain Monte Carlo method and a probability risk assessment approach) were combined to assess the shelf-life of an in-pack heat-treated, low-acid sauce intended to be marketed under chilled conditions. From a safety perspective, the product and process design for the chilled sauce was focused on the spore forming micro-organism Bacillus cereus. Different scenarios of time/temperature profiles in the food supply chain from manufacture up to the consumer were analysed in terms of growth of B. cereus (growth rate and lag phase) and of the consequence of this on the shelf-life. The end of the shelf-life was considered to be the time at which B. cereus reaches a concentration of $10^5$ cfu g⁻¹. For example, we have found equivalence in term of model output between scenarios in which the temperature in both retail and at the consumer’s home was below 6°C for 60 days, below 8°C for 28 days, and below 10°C for 17 days. These results can be used to support decisions relating to new product design, such as maximum shelf-life, target markets and labelling.

INTRODUCTION

Several governments and international organisations such as WHO and FAO have started developing probabilistic assessments of the risks posed by particular micro-organisms in foods on the market. These assessments involve predictive microbiology and microbiological risk assessment techniques. The problems tackled are very complex, since the risks are determined by a multitude of different factors including the micro-organism, the food supply chain and the consumer. Despite this complexity, the risk assessment approach has been able to provide risk managers with key information to help them decide on the necessity and suitability of risk intervention methods. Full-blown governmental risk assessments can be very resource intensive and often require advanced levels of skills and expertise, in areas such food microbiology, epidemiology and modelling. However, in specific applications (e.g., deciding on the suitability of the design of a new product before it is marketed) some form of probabilistic risk assessment approach may be helpful in an industrial context, when it can provide new relevant insights. In the current paper, this possibility is explored for a new low-acid sauce, which is to be heat-treated in pack, during manufacture and marketed in a chill chain for safety.

Our risk assessment approach comprises of four steps:

- Identification of the micro-organism(s) of concern (hazard identification)
- Development and running of a suitable exposure model
- Defining and validating the application domain of the model
- Applying the results to make safety recommendations.

Details regarding the models and their input values are specified below.
MODEL INPUTS

Hazard Identification

The product is a sauce with a pH around 6.0. The thermal process (90°C for 10 min) and the storage temperature (chilled storage) are the primary means of controlling bacterial hazards. For a safety perspective, the thermal process is considered sufficient to control non-proteolytic *Clostridium botulinum* but not *Bacillus cereus*. Since some types of *B. cereus*, i.e. the psychrotrophic stains, are able to grow below 8°C, this micro-organism was identified as the hazard of concern for which the product and process design should assure adequate control. The presence of large numbers of *B. cereus* (greater than 10^6 organisms g^-1) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health (FDA 2005). A level 1 log below this level (i.e. 10^5 cfu g^-1) was chosen as the cut-off level for the shelf-life.

Post-process Prevalence and Contamination Level

In a preliminary study, the prevalence rate and contamination level of psychrotrophic and mesophilic *B. cereus* spores, surviving the thermal process, was established (data not shown). This piece of work was based on both *B. cereus* heat resistance (more than 20 references compiled) and validated by comparison with levels of contamination reported in different heat treated foods (te Giffel et al., 1996; Choma et al., 2000; Nauta 2001). The prevalence rate was estimated to be 11% and 49% for surviving spores of psychrotrophic and mesophilic strains, respectively. The levels at which spores of both types of strains survived were estimated to be described by a Betageneral distribution with the shape parameters, $\alpha_1$ and $\alpha_2$, and the minimum and maximum values, min and max. For the psychrotrophic strains, $\alpha_1 = 1.56$, $\alpha_2 = 3.28$, min = -2.40, max = 2.24 ; for the mesophilic strains $\alpha_1 = 1.59$, $\alpha_2 = 1.91$, min = -2.40, max = 2.60.

Growth Model: Growth Rate and Lag Time at Low Temperatures

The preliminary and secondary models used to predict the bacterial quantity versus temperature, are the widely used exponential (Eq. 1) and square root (Eq. 2 and 3) models:

\[ \ln(N_t) = \ln(N_0) + \mu \cdot (t - \lambda) \quad \text{Eq. 1} \]
\[ \sqrt{\mu} = b \cdot (T - T_{\text{min}}) + \epsilon_1 \quad \text{Eq. 2} \]
\[ \frac{1}{\sqrt{\mu}} = c \cdot (T - T_{\text{min}}) + \epsilon_2 \quad \text{Eq. 3} \]

$N_t$ is the population size at time $t$ ($t>\lambda$), $N_0$ the post-process population size at $t=0$, $\mu$ the specific growth rate (h^-1) and $\lambda$ the lag phase duration (h). $T_{\text{min}}$ is the minimum growth temperature (°C), $T$ the current temperature (°C) and $b$ and $c$ two scaling parameters. As the secondary growth models (Eq. 2 and Eq. 3) were empirical, the model imprecision terms, $\epsilon_1$ and $\epsilon_2$ were also included in the comprehensive exposure model.

These equations have been used earlier in risk assessments of *B. cereus* (Zwietering et al., 1996; Nauta et al., 2003). After a heat treatment the bacterial spore “recovery” time, may be extended due to the pre-germination time and the time prior to spore conversion into a vegetative cell (Barker et al. 2004; Leguerinel, Quimper University, Personal communication). This was considered in the modelling.

Regarding model parameter estimation, the approach taken before by Pinon et al. (2004) and Membré et al. (2004) was used: when parameters could be considered as independent of the food (e.g. $T_{\text{min}}$), data collected in different food/media were used while challenge-tests were preferred for parameters assumed to be food specific (e.g. $b$ and $c$).

Parameters $T_{\text{min}}$, $\epsilon_1$ and $\epsilon_2$ were estimated using literature data (Griffiths and Phillips, 1990; Meer et al., 1991; Dufrenne et al., 1994; Dufrenne et al., 1995; Valero et al., 2000; Borge et al., 2001; Membré et al., 2005) and ComBase (http://wyndmoor.arserrc.gov/combase/).
The challenge-tests carried out on the sauce product, were done at constant temperatures (5, 7 and 10°C) for at least 4 weeks. A Bayesian Markov Chain Monte Carlo method was employed to estimate the model parameters. This method was chosen for its flexibility in parameter and random deviate analysis (Pouillot et al., 2003) and also for its ability to integrate prior expert knowledge. To establish the separation between psychrotrophic and mesophilic strains of *B. cereus*, and especially to estimate the lower limit of temperature below which no growth is expected ($T_{min}$), internal microbiology experts were consulted. As for prior distributions, $T_{min}$ values for the psychrotrophic and mesophilic strains were assumed to be in a range of 3–5°C and 7–9°C, respectively. To perform the Bayesian Markov Chain Monte Carlo analysis, WinBug (version 1.4, Medical Research Council, UK) was run. Results are presented in Figures 1 and 2.

**Temperature and Time in the Supply Chain**

Based upon preliminary work, we have considered that storage at pre-retail, retail and by the consumer were the key steps in the food supply chain impacting on shelf-life. Therefore realistic time/temperature scenarios needed to be established for these steps in relation to the intended shelf-life. For example when the shelf-life was expected to be 28 days, the time spent at pre-retail (factory, transport and distribution centre), at retail (local market, supermarket) and at the consumer were described by Uniform distributions with $min = 1$ day, $max = 7$ days; $min = 1$, $max = 28 - 7 - 1$; and $min = 1$, $max = 28 - pre-retail time - retail time$, for time spent at pre-retail, retail and consumer, respectively.

The temperature at pre-retail, based on internal supply chain expertise, was chosen as a Triangular distribution ($min = 1$, likely $= 3$, $max = 5°C$). We assumed that the temperature inside the food was equal to the temperature of the refrigerator.

Concerning retail and consumer refrigerators, the distributions were fitted using unpublished survey data in the European market (Fig. 3). The means were estimated to be 5.2 and 6.3°C, and the 99th percentile to 12.9 and 10.5°C, respectively. Based on expert opinion of local conditions and data, the temperature of food inside retail refrigerators was considered as varying by $±2°C$. These fluctuations corresponded to variations between food positions in the refrigerator or within the cooling cycle process. They were fitted by a Normal distribution ($mean = refrigerator temperature$; $Sd = 0.85°C$). The same fluctuations were introduced for consumer refrigerators, using an approximate $±1°C$ change (Normal distribution with a mean $= refrigerator temperature$; $Sd = 0.42°C$). These small fluctuations were considered as variability and were systematically integrated with the exposure model, whatever the time/temperature scenario.

**MODEL OUTPUTS AND VALIDATION**

Model outputs were the probability to reach a concentration of $10^5$ cfu g$^{-1}$ *B. cereus* in the product at a certain shelf-life. This level was assumed to signify the maximum shelf-life on the market. The probability of the bacterium to develop to this level at the end of the shelf-life can be interpreted as the failure rate of packs not being suitable to be marketed. Results were obtained after a 100,000 iteration run (@Risk, Palisade corporation, Newfield, USA). Simulations were run for both psychrotrophic and mesophilic strains and for different time/temperature profiles. To include the prevalence rate, the probability to obtain the tolerance limit was calculated using the Bayes’ theorem:
\[
\begin{align*}
\text{with } & \Pr\{\text{presence of bacteria}\} = "\text{Prevalence"}\ \\
\text{and } & \Pr\left\{ N_i > 10^5 \right\}_{\text{in absence of bacteria}} = 0 \\
\Pr\left\{ N_i > 10^3 \right\} & = \Pr\left\{ N_i > 10^5 \right\}_{\text{in presence of bacteria}} \cdot \Pr\{\text{presence of bacteria}\} \\
& \quad + \Pr\left\{ N_i > 10^5 \right\}_{\text{in absence of bacteria}} \cdot \Pr\{\text{absence of bacteria}\} \\
\text{then } & \Pr\left\{ N_i \leq 10^5 \right\} = 1 - \Pr\left\{ N_i > 10^5 \right\}_{\text{in presence of bacteria}} \cdot \text{Prevalence}
\end{align*}
\]

To obtain a shelf-life of 28 days, the sensitivity analysis performed for both psychrotrophic and mesophilic strains indicated that the three key inputs were the temperature at the retailer, the growth rate model error term, \( \varepsilon_1 \), and the refrigeration temperature at the consumer (Fig. 4).

- For example, with psychrotrophic strains, when Monte Carlo simulations were run with \( \varepsilon_1 \) fixed at its 99th percentile, the bacterial concentration was estimated to be 7.4 log cfu g\(^{-1}\) (95% limit) after 28 days.
- If the retail or the consumer temperatures were constantly at their 99th percentile (12.9°C or 10.5°C, respectively), the bacterial concentration of psychrotrophic strains was estimated to be 8.6 or 6.4 log cfu g\(^{-1}\) (95% limit).
- The temperature at pre-retail, varying from 1 to 5°C, had very little effect on the concentration of psychrotrophic strains of \( B.\ cereus \) at the end of the shelf-life and no effect on the concentration of mesophilic strains.

In Fig. 5, the impact of the combination of model imprecision terms \( \varepsilon_1 \) and \( \varepsilon_2 \), at low temperatures (7.0 and 8.0°C) on the final contamination level is illustrated with the psychrotrophic strains. When the temperature is kept at exactly 7°C, assuming that the post-cooking contamination is for instance 10\(^1\) cfu g\(^{-1}\), there is a probability of 0.83% to obtain 10\(^5\) cfu g\(^{-1}\) or more, after 28 days (based on a 10,000 simulation run). Model error is currently considered as a source of uncertainty (lack of knowledge) in a risk assessment output, while the distribution of the model parameter \( T_{\text{min}} \) is mainly driven by the strain variability (Pouillot et al., 2003). To reduce the uncertainty in our exposure model output (as illustrated in Fig. 5), the growth model will be consolidated by performing additional challenge tests. Experiments are in progress.

To deal with the variability brought about by the temperatures of retail and consumer refrigerators, shelf-life was estimated at different temperature values. For example, there was the same probability (estimated to be 1 in 10,000) to obtain more than 10\(^5\) cfu g\(^{-1}\) when the food is kept in retail and consumer refrigerators with mean temperatures below 6°C for 60 days, 8°C for 28 days or 10°C for 17 days.

The probabilistic assessments of the risks posed by particular microorganisms in food have already been developed. The originality of this study is its application within the food industry. Indeed, here, several kinds of modelling techniques were combined:

- predictive microbiology to integrate the temperature effect on bacterial recovery and growth,
- Bayesian Markov Chain Monte Carlo method to estimate the model parameters, using different sources of information (literature, challenge-tests, expert opinion),
- Probabilistic risk assessment approach to generate different scenarios of temperature/shelf-life and to estimate the probability of having a food product contaminated with more than 10\(^5\) cfu g\(^{-1}\) under these scenarios.
- Sensitivity analysis to identify the key inputs in our model and to explore how the model could be consolidated.

More traditionally decisions on the shelf-life of a chilled product are often based on multiple challenge tests at low temperatures. The relative complexity of the modelling techniques needs to be compared to the time/resource required for performing challenge-
tests. Likewise, interpretation of a model output (e.g. interpretation of probability) requires more effort than interpretation of a challenge-test. But the relative simplicity of challenge-test data offer belies the complexity of the supply chain it is attempting to simulate.

Then, for practical application, the modelling approach proved valuable for several reasons. These include:

- By introducing input parameters as distributions and by deploying the Monte Carlo method for probabilistic risk assessment, the model output is also a distribution of values and not a single value. The probability curve represents a more realistic situation of the likely microbial dynamics in the supply chain than an absolute value would (whether e.g. mean, 95th percentile or worst case).
- It enables easy running of different time/temperature profiles in the food supply chain, comparing the different outcomes with their specific uncertainties and/or variabilities. Segregation of the impact of different scenario’s can give a sense of the robustness and adequacy of the product and process design in relation to the intended shelf-life.
- Each scenario can be analysed to understand the magnitude of impact of individual factors on the outcome, for instance by using the sensitivity analysis technique.
- Knowing the relative importance of particular factors in determining the shelf-life can help in identifying which points or conditions in manufacture may be critical for pathogen control and thus should be carefully implemented in the manufacturing operation.

Literature Cited


Figures

Fig. 1. Distributions obtained for the growth rate and the lag time duration, at 7 (black line) and 10°C (grey line) for psychrotrophic strains.

Fig. 2. Distributions obtained for the minimum growth temperature for both psychrotrophic and mesophilic strains.
Fig. 3. Mean temperature of retail (closed symbols) and consumer (open symbols) refrigerators in European market. Data and fitted distributions.

Fig. 4. Tornado graphs to illustrate how the outputs are sensitive to the input variations (in a range from 1st to 99th percentile). a. Psychrotrophic strains. b. Mesophilic strains. Pre-retail and retail time based upon a 28-day shelf-life.
Fig. 5. Impact of the model error terms $\varepsilon_1$ and $\varepsilon_2$, at low temperatures (7.0 and 8.0°C) on the final contamination level of psychrotrophic strains of *B. cereus* (expressed in log$_{10}$ cfu g$^{-1}$ in the isocontour lines). Eq. 1 with log$_{10}(N_0)=1$, t=28 d.