

Application of Monte Carlo Simulation in Industrial Microbiological Exposure Assessment

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1. Introduction

1.1 Food safety systems: HACCP and risk analysis

Nowadays, modern societies pay great attention to food safety. For this reason, tools such as Hazard Analysis and Critical Control Points (HACCP) and Risk Analysis (RA) have been developed, helping the production of safer foodstuffs and, consequently, reducing the number of foodborne illnesses. Nevertheless, there are certain differences between HACCP and RA, HACCP system is the main tool in evaluating and controlling foodborne hazards in food industries while risk analysis demonstrate to be an effective tool in designing, developing and evaluating control measures protecting public health inside a country or region. In addition, RA has been used for seeking solutions in commercial litigation as an objective tool to assess the risk that a food has to the consumer (Zwietering & Nauta, 2007). HACCP systems do however have some limitations, such as the inability to be linked with public health goals and the incapability to deal with the inherent variability in food systems due to its qualitative nature (Buchanan & Whiting, 1998; Hoornstra et al. 2001).

Risk analysis is a complex process consisting of three interconnected components: risk assessment (scientific component), risk management (legal component) and risk communication. Risk assessment consists of four steps, *hazard identification* where the hazard is related to the onset of illness by using data from foodborne outbreaks caused by food contaminated with the hazard, as well as its taxonomy and virulence factors associated with the development of the disease. Having identified the hazard, a *hazard characterisation* is carried out which identifies the aetiology of the disease caused by the biological agent, the influence of different subgroups on the disease symptoms and the dose-response relationship. The third step is the *exposure assessment* which deals with the ecology of the biological hazard, the critical steps of the production chain in which the hazard could be present or proliferate, and the control measures which determine the influence of environmental factors and process conditions on the survival of the biological agent. Finally, all the information gathered in the previous sections is used to produce an estimation of the

risk, known as *risk characterisation*, expressed as the probability that a population acquires the disease by the consumption of the food contaminated with the hazard.

Risk assessment has a qualitative or quantitative nature depending on the availability of data. The qualitative assessment is the most widely used because of the lack of data on consumption pattern, dose-response models, initial contamination, and survival of the microorganism after treatment and until the time of consumption. In this case the magnitude of the risk can be described as insignificant, low, medium and high.

The quantitative microbial risk assessment (QMRA) is more complex and it is based on the availability of specific quantitative data concerning the prevalence of the hazard in the product under study at different steps of the process chain, as well as, the necessary dose to produce a response in the host (dose-response relationship) and the use of mathematical models to characterise that response.

The limitations of HACCP commented earlier can be overcome by combine it with risk assessment providing to the HACCP system with quantitative data for CCP establishment. Nevertheless, the use of the risk assessment in the industrial environment (IMRA) is still an underdeveloped tool.

1.2 The exposure assessment as part of risk assessment

The exposure assessment as a part of the risk assessment process estimates the presence of pathogens or microbial toxins and the probability that they will be present in the product at the time of consumption or at a given moment of the production process (process level for example), which is known as "farm to table" framework. The exposure assessment takes into account some factors such as the frequency of contamination caused by the pathogen or its level in the food during the shelf-life. At the same time, these factors can be influenced by:

- characteristics of the pathogen and its environment.
- microbial ecology of food and the initial contamination of raw material.
- level of hygiene control, methods of processing and preservation, packaging, distribution and storage.
- preparation of food: cooking, holding times, etc.
- consumption patterns.

All this make the levels of microbial pathogens in foods very dynamic. Therefore, the exposure assessment should describe the microorganism behaviour from production to consumption. In this sense, it is necessary to have instruments to facilitate the completion of the quantitative exposure assessment. Useful tools to develop a proper quantitative exposure assessment are predictive microbiology and the Monte Carlo simulation.

1.3 Predictive microbiology and mathematical models in exposure assessment

Predictive microbiology is a discipline that combines elements of microbiology, mathematics and statistics to develop models that describe and predict the behaviour of microorganisms under certain experimental conditions. Specifically, it is based on the idea that organisms have reproducible behaviour and can be described as a function of different variables through a model. A model can be defined as: "a simplified representation, which includes key aspects of existing systems, which can be used for predictive purposes and control" (Eykoﬀ, 1979). Predictive microbiology is essential to perform a quantitative exposure assessment because through it and using a simulation procedure, changes in the number of microorganisms in a production line can be estimated providing an assessment of exposure

to a particular pathogen. Traditionally, inactivation and growth data have been adjusted to deterministic models obtaining in this way the kinetic parameters. However, the development of probabilistic models that actively consider product (ingredients or formulation) and process variability including the whole distribution (from minimum to maximum with all modes and percentiles) has gained importance in recent years and are essential in conducting risk assessment studies (Baert et al. 2009; Buchanan & Whiting, 1998; Hoornstra et al. 2001; Nauta, 2002).

1.4 Simulation: an essential tool in exposure assessment.

The simulation has been an important tool in industrial design tasks regardless of the application field. Although it has been considered the last alternative in the absence of data, recent advances in software have made it one of the most used and accepted tools in analysis and research. Simulation using computer allows us to replicate experiments or recreate scenarios using selective changes in the parameters or operating conditions. Correlations between sequences of random numbers to improve the statistical analysis of simulation output can be also entered. However, the simulation is an imprecise technique that provides only statistical estimates, not exact results, likewise, only compare alternatives rather than to generate an optimal value.

Because the sample of a particular distribution involves the use of random numbers, stochastic simulation is sometimes called **Monte Carlo simulation**. This method is one of the most powerful and used to analyse complex problems which may occur on sceneries related with food safety and is particularly appropriate in systems with many degrees of freedom (Larcher, 2006). It presents the advantage of being broadly applicable and relatively easy to use. It must be remembered that the Monte Carlo simulation is a computer-based technique that allows that variations to the input variables are propagated through inactivation, growth or germination mathematical models, which provides information about variation in the final result. In addition, Monte Carlo simulation allows also the possibility of carrying out a sensitivity analysis of all parameters involved in the model. The use of the Monte Carlo simulation, where input parameters are described as frequency distributions, is an example of stochastic or probabilistic analysis (van Gerwen and Gorris, 2004). However, the application of the Monte Carlo simulation at a process level exposure assessment study is still scarce and only a few studies are available (den Aantrekker et al. 2003; Ferrer et al. 2006, 2007; Pina-Pérez et al. 2010; Sampedro et al. 2010).

The technique can be implemented in many ways, the easiest option is an “add-in” for Microsoft Excel being the most commonly used @Risk (Palisade Corporation, 2004) and Cristal Ball (Decissionearing, 2005). Besides, there is also the possibility of using the numerical analysis software Matlab (The Mathworks Inc, 2006).

In this chapter a description of the Monte Carlo technique applied to industrial exposure assessment is given through a simple illustrative example of modelling the germination of *Bacillus cereus* spores and the simulation of the number of spores that can germinate at a given time of a process, considering different scenarios.

2. Case study: Exposure assessment of *Bacillus cereus* in liquid egg

In the previous sections it have been discussed the possibility of using predictive microbiology and the Monte Carlo simulation for assessing the level of exposure of a hazard. In most cases, sporulated microorganisms or vegetative cells are used to conduct the

study. However, when sporulated microorganisms are used, germination has not been considered as a step and therefore there are only a few models describing this cellular event. The incorporation of information on germination as well as environmental factors that condition such a germination process, can be very valuable in estimating the risk associated with consumption of foods that can be contaminated with sporulated organisms.

In the example given in this chapter, the germination process of *Bacillus cereus* has been mathematically modelled and simulated in liquid whole egg. The Weibull distribution function was applied to model the germination process (Equation 1) by using a nonlinear regression performed with the SPSS statistic software (The Apache Software Foundation, 2000) and the Solver add-in option in Excel (Microsoft Corporation, 2003). In the last decade, the Weibull model has been often applied in food technology mainly in those cases where the inactivation of microorganisms did not follow the log-linear model. It has been used to describe degradation kinetics of food, those events can be considered failures in the system after being subjected to some stressful conditions during a certain time (Garcia, 2004). It has also been used to describe the kinetics of hydration of cereal used for breakfast (Machado et al., 1998) and thin-layer drying of cereals, grains and fruits (García, 2004). In the area of microbiology it has been applied to describe the survival kinetics of different microorganisms (Fernandez et al., 2002; Ruiz et al., 2002) to follow the combined effects of pH and temperature on the thermal resistance of *B. cereus* (Collado et al., 2003) and to compare the thermal resistance of *B. subtilis* (Jagannath et al., 2005). In the case of nonthermal technologies, it has been used to describe the inactivation kinetics of *E. coli* by pulsed electric fields processing, or by high pressure processing (Rodrigo et al., 2003), *L. plantarum* (Sampedro et al., 2006) and *C. sakazakii* (Pina-Perez et al. 2007a, Pina-Pérez et al., 2007b), among other applications.

$$\frac{G_t - G_0}{G_\infty - G_0} = 1 - e^{-\left(\frac{t}{\alpha}\right)^\beta} \quad (1)$$

where G_t is the germination at time t of the experiment, G_0 is the initial germination, G_∞ is the germination at the equilibrium point, α is the scale parameter, and β is the shape parameter of the Weibull distribution respectively.

Considering the results of modelling the germination, a simulation that considered the effect of induced germination and subsequent pasteurisation on the final population of *Bacillus cereus* in liquid egg was carried out by using equation 2. For that purpose, the Monte Carlo simulation was applied using the numerical analysis program Matlab 7 (The Mathworks Inc, 2006). It was considered that the time and the parameters β and α of the Weibull distribution followed a uniform distribution.

$$\text{Log}N = \text{Log}N_g - \frac{t}{D_R * 10^{\left(\frac{T_p - T}{z}\right)}} \quad (2)$$

where N is the final population of microorganisms, N_g indicates the germinated population of *B. cereus*, t is time, D_R is the D value at 65 °C, T_p is the temperature of pasteurisation, T is the treatment temperature and z is the sensitivity of microorganism to the variation of temperature. In the simulation model, the variables t , D_R , T_p , z , are not considered deterministic but probabilistic. Therefore, they do not enter the model as exact values but as probability distributions, which for simplicity are considered as uniform.

2.1 Modelling the germination of *Bacillus cereus*

Figure 1 shows the response of *Bacillus cereus* in liquid egg, after applying three different concentrations of inosine as germinant (10, 5 and 1 mM).

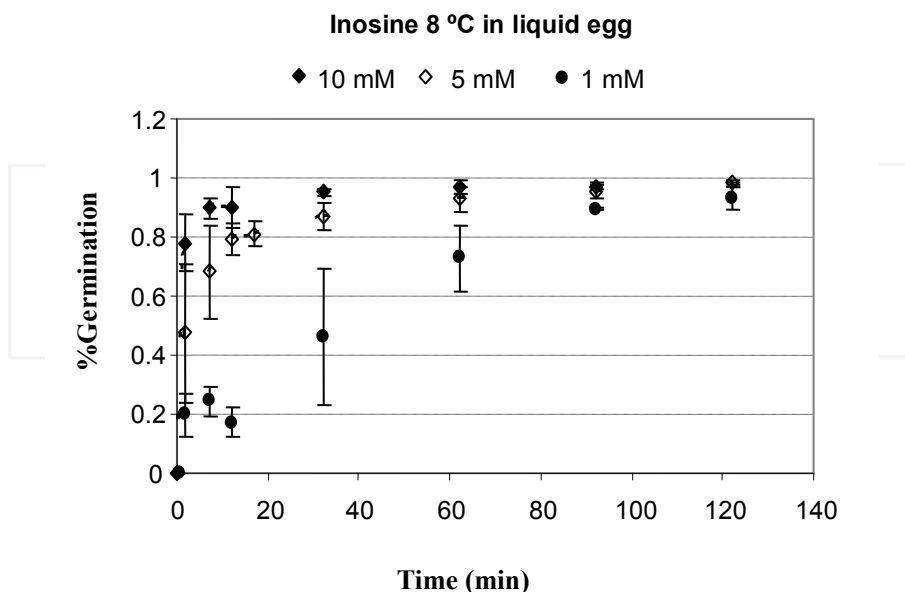


Fig. 1. Evolution of the germination of *B. cereus* at 8 °C in liquid egg using inosine as germinant.

It was found that at the temperature of 8 °C the endospores germinated well and after 122 minutes, germination was around 93 and 98%. On the other hand, a clear effect of the concentration of inosine on the germination response was observed. As increasing the concentration of germinant, germination was faster, existing significant differences between the three concentrations used in the study (10, 5 and 1 mM) according to Tukey's test (Sig <0.005).

From literature studies (Fernández et al. 2001) it can be concluded that endospores of *B. cereus* in liquid egg can survive the pasteurisation process thus can cause serious health problems. Since germination is considerable even at refrigeration temperatures, it could be convenient to apply this germination treatment previous to thermal pasteurisation. This technological procedure can help in reducing the number of endospores present in liquid egg. Therefore, the risk of illness caused by *B. cereus* could be reduced.

Figure 2 shows the experimental data fitted to the Weibull model according to equation 1.

As a measure of goodness of fit, the mean square error (MSE), the adjusted regression coefficient (R^2) and accuracy factor (A_f) were used. The MSE reports the error variance, so that the lower the value the better the model fits the data. With respect to R^2 , the higher the value the better the model describes the data. Finally, the accuracy factor A_f provides a measure of the average difference between the values obtained experimentally and the values predicted by the model. The closer the value to the unit the better is the description of experimental data made by the model. Table 1 shows the values of MSE, R^2 and A_f obtained for each non-linear regression.

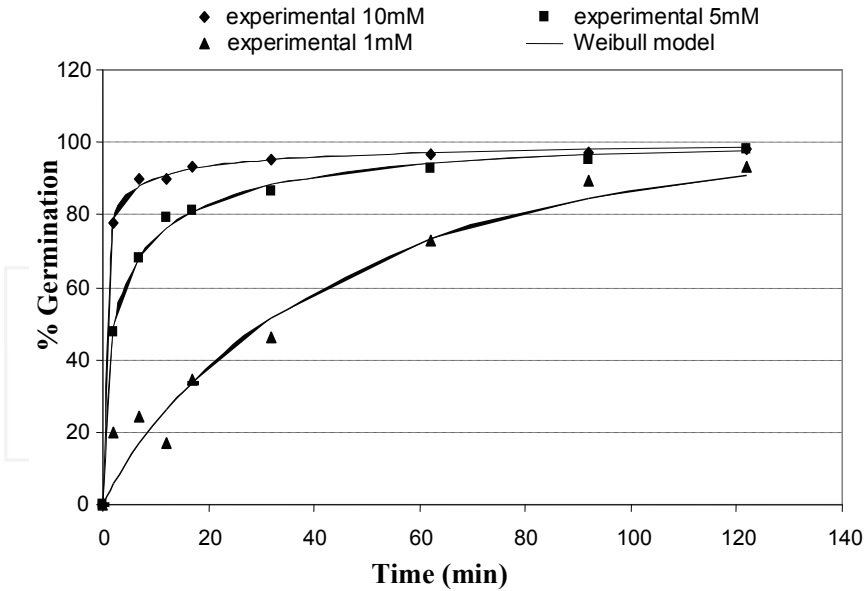


Fig. 2. Weibull fitted model to the germination data of *B. cereus* in liquid egg at 8°C.

Germinant concentration	α	β	MSE	R ²	A _f
10 mM	0,331±0,243	0,243±0,136	0,890	0,998	1,007
5 mM	7,521±0,948	0,570±0,072	2,779	0,996	1,013
1mM	45,802±7,194	0,920±0,164	55,021	0,943	1,152

Table 1. Values and confidence intervals of the different parameters after fitting experimental data to the Weibull distribution function.

The results in Table 1 indicated that the model was adequate, especially for cases where the concentration of inosine was high. R² values obtained were close to 1, corresponding to their highest values with the lowest MSE and A_f. A low value of MSE means a small error of variance, and therefore, the model fitted well the experimental data. The value of A_f indicated that, on average, the prediction made by the model differs from the experimental data from 0.7 to 15% for 10 to 1 mM respectively. On the other hand, the value of *a* could be considered as the kinetic constant, which could be related to the rate at which germination occurs; the higher value of *a*, the slower the germination response. In fact, it can be seen that *a* increased with decreasing the concentration of germinant. Therefore, a high value of *a* would indicate a lower germination rate.

The β parameter describes the shape of the curve. Thus, β values greater than 1 ($\beta > 1$) indicate the presence of shoulders, while β values lower than 1 ($\beta < 1$) notes the appearance of tails. When β is equal to 1 ($\beta = 1$), the Weibull distribution reduces to a first-order kinetic model. In this case study, all β values were lower than 1. As far as germination was concerned, β could be related with the saturation of the receptor to the inosine germinant. In

this context, the highest germination was achieved for smaller β values, reaching at the end of the study an germination equilibrium point. Contrarily, the curves shown higher β values corresponded to cases where final germination obtained was lower.

It can be concluded that the Weibull model fitted satisfactorily the experimental data, rendering predictive curves from which predictions could be obtained for a determined amount of treatment time. However, despite its usefulness, this is not sufficient to carry out an exposure assessment.

2.2 Monte Carlo simulation

The industrial exposure assessment requires a model that relates the probability distribution of the population at the end of the production process with the probability distribution of the population present at the beginning of the process. For this reason, a Monte Carlo simulation for each germinant concentration was conducted.

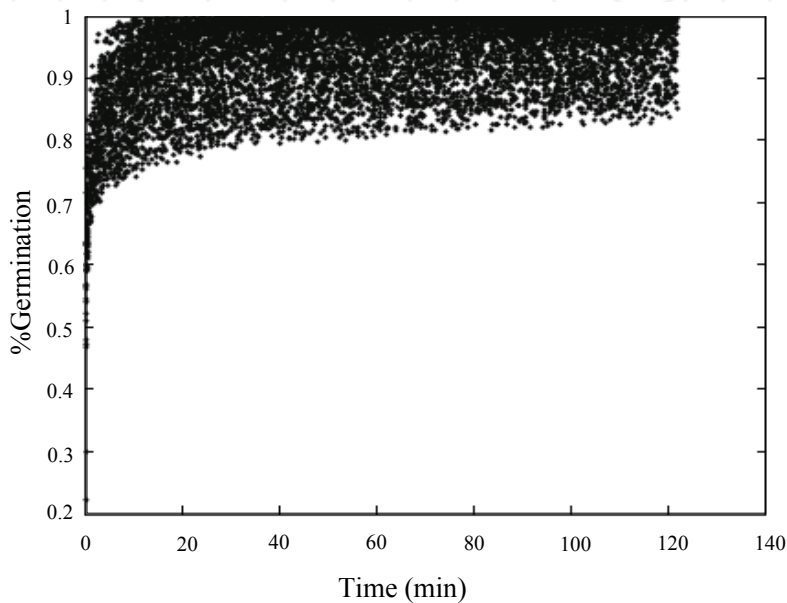


Fig. 3. Simulation of germination of *B. cereus* with 10 mM inosine at 8 °C in liquid egg.

The first variable considered by the simulation model was the initial concentration of microorganisms. This concentration was considered 10^6 cfu/g based on a study provided by producers in the different stages of an industrial production process. Specifically, it was studied the steps that could influence the presence of *B. cereus* in the final product, concluding that there was a risk of microbial contamination in the following operations: egg reception, cracking, separation and storage. In the case of the reception of eggs, the risks lied on the bacteria present both outside and inside the egg shell. Additionally, during the breaking and cracking, separation and storage, microbial concentration could increase in the liquid egg due to the contact with dirty egg-shells or contaminated surfaces and equipments.

Probability distributions of other variables to be considered in the germination simulation were introduced in the exposure assessment model. It was considered that the time, the

shape and scale parameters (α and β) and the germination rate followed a uniform distribution. That is, each value was associated with the same probability of occurrence in a known interval. In the case of the parameters a and β , that interval was calculated from the values provided by the model fit and their confidence interval.

The results after a simulation with 10,000 iterations are shown below. The curves obtained by simulation were consistent with those experimentally obtained. Figure 3 shows the result of germination simulation in the case of adding 10 mM of inosine. The possible germinative scenarios are contained in the cloud of points showed in the figure. The results for the other cases studied (1.5 and 1 mM), are presented in Figures 4 and 5.

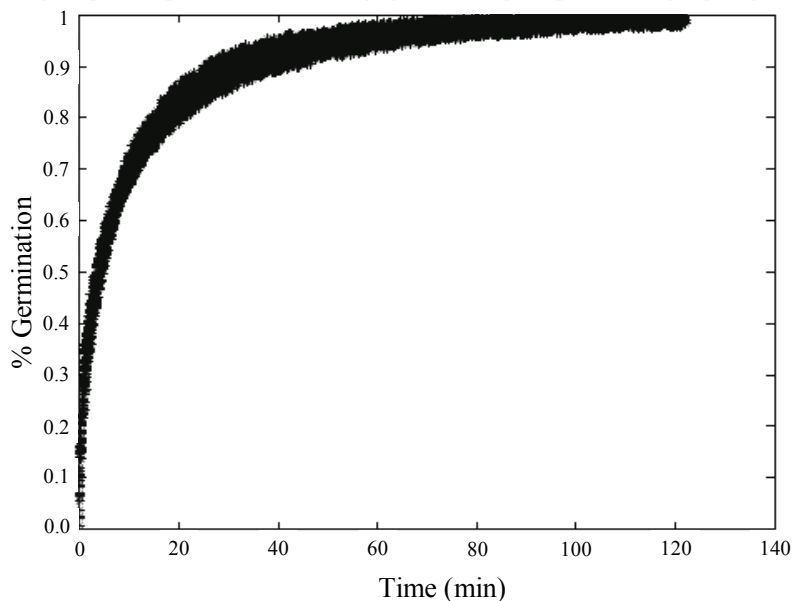


Fig. 4. Simulation of germination of *B. cereus* with 5 mM inosine at 8 °C in liquid egg.

The introduction of these results within a thermal treatment model is useful because it allows calculate the probability of occurrence of *B.cereus* between certain limits in the final product. Thus, it was calculated the probability that the liquid egg contained a number of microorganisms after the germination and the subsequent pasteurisation of samples (Table 2).

N° ufc/g	Probability Inosine 10mM	Probability Inosine 5mM	Probability Inosine 1mM
Between 0-1	100%	100%	100%
Between 10 ⁵ -5x10 ⁵	0	0	0

Table 2. Probability that final product being contaminated with a certain number of microorganisms after receiving a standard pasteurisation process (10⁵ is considered the infective dose).

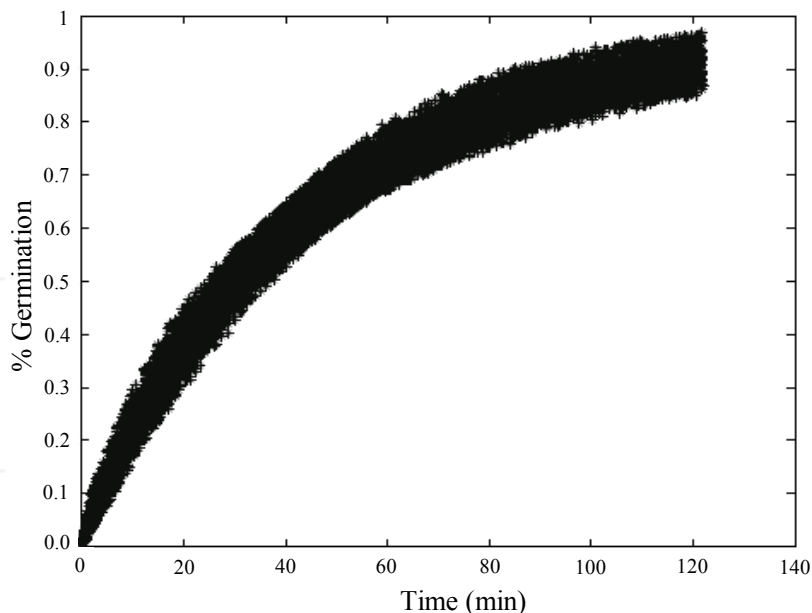


Fig. 5. Simulation of germination of *B. cereus* with 1 mM inosine at 8 °C in liquid egg

These results indicate that induced germination followed by a pasteurisation would yield a final product ready for storage and packaging, virtually *B. cereus* free. This is important for two reasons. First, it ensures product safety in the absence of cold chain due to a breakdown during storage, packaging, distribution and consumption. Secondly, the probability of rejection of contaminated liquid egg batches is considerably reduced because the maximum level allowed by manufacturing companies, cream caramel producers, is not attained. It should be noticed that most of the liquid egg produced is employed as raw material for manufacturing of desserts, e.g. cream caramel, with their own very restrictive internal quality standards, requiring absence of *B. cereus* in 400 g of liquid egg.

It is generally accepted that the infective dose by *B. cereus* is 10^5 cfu/g (Doyle, 2004). In this case, the probability of liquid egg could be contaminated at that level is practically zero, which would be within safety limits for its commercialisation.

What would happen with the final population of *B. cereus* in liquid egg if the scenario changes, for example, the occurrence of a failure in the pasteurisation process? Let us see the result considering the following scenario: the liquid egg receives a thermal treatment at 57°C, rather than the mandatory at 65°C or higher. Table 3 shows the results obtained.

N° Ufc/g	Probability Inosine 10mM	Probability Inosine 5mM	Probability Inosine 1mM
Between 0-1	0	0	0
Between 10^5 - 5×10^5	15.40%	32.51%	51.80%

Table 3. Probability of final product contamination after a failure in the pasteurisation process (10^5 is considered the infective dose).

After comparing the results of Table 3 with those presented in Table 2 we can conclude that when a failure in the pasteurisation process was produced, and the liquid egg was treated at 57 °C, the probability of finding *B.cereus* in the final product, in a concentration that can produce illness, would be considerably higher.

In resume, the result of the simulation shows that as the amount of germinant is reduced the probability of finding microbial counts within the range 10^5 - 5×10^5 (infective dose) increases. The result is consistent with the kinetic results since the 1 mM concentration produced less germination (Figure 1) and consequently more endospores will survive the thermal treatment at 57 °C. It would be advisable; therefore, the use of 10 mM as optimal concentration of germinant together with good hygienic practices during the production to prevent for a large number of *B. cereus* spores in the final product even if a failure in the pasteurisation process occurs. This simulation result warns about the need of a proper maintenance service of the pasteurisers and indicates that pasteurisation is a Critical Control Point in the manufacture of liquid egg.

It is important to note that the above findings are influenced by the boundary conditions applied. In fact, the simulation was performed by considering a very high initial contamination: 10^6 cfu/g (the worst scenario). In this sense, the simulation also demonstrates that the implementation of HACCP is a crucial activity to maintain the safety of the product, even when a failure in the process is produced. For that, the introduction of the Monte Carlo simulation by using an Industrial Risk Assessment approach is of great interest for the industry.

3. Conclusions

The results obtained with this simulation by using Monte Carlo are very useful since they give a quantitative estimate of the exposure assessment at the end of the pasteurisation process. This information can be crucial in establishing the management options for other operations that follow the pasteurisation process until the product reaches the consumer. It is important to point out that the simulation of the germination can be implemented as part of a process that simulates the entire production chain through the use of modules for each one of the stages, approach known as the Modular Process Risk Model (MPRM).

Achieving a MPRM is a complex task due to the many variables that may affect the evolution of the microbial counts. Basically, the MPRM is characterised by identifying the basic operations (modules) that comprises the food production and the conditions that determine the presence of the microorganism in those modules, and later on use mathematical models that explain adequately the situation under study. Finally, additional information such as the dose-response relationship, consumption patterns and handling of food in the household or the establishment of a given population is needed since the level of exposure depends not only on what happens during the process but also on the consumer behaviour. Taking into account the previous considerations, the usefulness of the simulation carried out in this case study is clear, since it makes possible its inclusion in a risk model with a modular structure, providing the basis for a quantitative industrial risk assessment.

This case study that simulates the germination of *B. cereus* for the subsequent inclusion in a heat treatment model, has led to different values of probability of having a certain number of microorganisms at the end of the pasteurisation process, considering different scenarios, assessing the exposure level to *B. cereus* in liquid egg. It has showed how the Monte Carlo Tool simulation is a tool to be considered in the food safety and decision making in an industrial plant, allowing management activities considering a quantitative approach.

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