Estimation and evaluation of management options to control and/or reduce the risk of not complying with commercial sterility

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ABSTRACT

In a previous study, a modular process risk model, from the raw material reception to the final product storage, was built to estimate the risk of a UHT-aseptic line of not complying with commercial sterility (Pujol et al., 2015). This present study was focused on demonstrating how the model (updated version with uncertainty and variability separated and 2nd order Monte Carlo procedure run) could be used to assess quantitatively the influence of management options. This assessment was done in three steps: pinpoint which process step had the highest influence on the risk, identify which management option(s) could be the most effective to control and/or reduce the risk, and finally evaluate quantitatively the influence of changing process setting(s) on the risk. For *Bacillus cereus*, it was identified that during post-process storage in an aseptic tank, there was potentially an air re-contamination due to filter efficiency loss (efficiency loss due to successive in-place sterilizations after cleaning operations), followed by *B. cereus* growth. Two options were then evaluated: i) reducing by one fifth of the number of filter sterilizations before renewing the filters, ii) designing new UHT-aseptic lines without an aseptic tank, i.e. without a storage period after the thermal process and before filling. Considering the uncertainty in the model, it was not possible to confirm whether these options had a significant influence on the risk associated with *B. cereus*. On the other hand, for *Geobacillus stearothermophilus*, combinations of heat-treatment time and temperature enabling the control or reduction in risk by a factor of ca. 100 were determined; for ease of operational implementation, they were presented graphically in the form of iso-risk curves. For instance, it was established that a heat treatment of 138 °C for 31 s (instead of 138 °C for 25 s) enabled a reduction in risk to 18×10^{-8} (95% CI = [10; 34] $\times 10^{-8}$), instead of 578×10^{-8} (95% CI = [429; 754] $\times 10^{-8}$) initially. In conclusion, a modular risk model, as the one exemplified here with a UHT-aseptic line, is a valuable tool in process design and operation, bringing definitive quantitative elements into the decision making process.

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1. Introduction

Commercial sterility of thermally processed food corresponds to the condition achieved by application of sufficient heat to render the food free from microorganisms capable of growing in the food under ambient storage conditions (Codex Alimentarius Commission, 1993a). Aseptic processing and packaging correspond to the plant operations aiming at filling a commercially sterile product into sterilized containers followed by hermetically sealing while preventing viable microbiological recontamination (Codex Alimentarius Commission, 1993b). Therefore, the critical steps when running an aseptic-Ultra-High-Temperature (UHT) line are the sterilization process (of both product and packaging) and

the filling operation; any intermediate step where air-recontamination might occur, such as post-process storage of the product in aseptic tank or post-sterilization storage of the packaging unit, has to be limited or even avoided (den Aantrekker et al., 2003). In parallel, the number of batches between cleaning-in-place operations, and, the cleaning procedures have to be well monitored to prevent biofilm formation (Marchand et al., 2012). In a previous study, a Quantitative Microbial Exposure Assessment (QMEA) model was built to estimate the risk of a UHT-aseptic line of not complying with commercial sterility (Pujol et al., 2015). The product was a milk-based product filled in 200 ml bottle; the model had nine different modules (one per key process step), from the raw material reception to final product storage, it included intermediate storages and covered sterilization of the product on one hand, and sterilization of the packaging on the other hand. The model settings corresponded to those currently run in several factory lines, i.e., they corresponded to actual and implemented factory control measures.

The model was developed for *Clostridium botulinum*, *Geobacillus stearothermophilus* and *Bacillus cereus*; *C. botulinum* was chosen as the pathogenic bacteria which has to be considered in priority in a risk assessment of aseptic-UHT-type products (Codex Alimentarius Commission, 1993a), *B. cereus* was included as a pathogenic bacteria commonly found in milk powder (Becker et al., 1994) and finally, *G. stearothermophilus* was taken into account because it can survive to very high heat treatment (Rigaux et al., 2013) and has been then often reported as responsible of ambient stable product spoilage (Denny, 1981; Ito, 1981; Prevost et al., 2010). More precisely, André et al. (2013) have determined that *Geobacillus* sp. was involved in 35% of non-stability cases of canned food. Also, Burgess et al. (2010) have pointed out that, in dairy factories, *G. stearothermophilus* bacteria are difficult to eliminate (as they are heat resistant spore formers) and tend to readily form biofilms.

Since its initial development, the model has been refined with the addition of uncertainty dimension to the probabilistic inputs. The probabilistic inputs were built using external sources (EFSA website, literature) or through expert elicitation session. Uncertainty and variability were separated using a second order Monte Carlo procedure as recommended and applied in food safety domain (Cummins et al., 2008; Pouillot et al., 2004; Vicari et al., 2007). The objective of the work reported in this paper was to suggest management options to control and/or reduce the risk of having a UHT-aseptic product not complying with commercial sterility. The methodology was carried out into three steps. First, the model was used to pinpoint which process step had the highest influence on the risk, and second to identify which management option(s) could be the most effective to control and/or reduce the risk. In a last step, the influence of changing process setting(s) related to this option(s) was evaluated quantitatively. This paper provides several examples of management options which enable the control and/or reduction of risk associated with an aseptic UHT dairy product line, and details the methodology carried out to identify and evaluate these options.

2. Materials and methods

2.1. Quantitative microbial exposure assessment model

The QMEA model, previously built in our research group with the help of factory experts, encompassed steps from the raw material reception to the end-product storage (Pujol et al., 2015). It included nine modules as described hereafter. The first two modules took into account the introduction of microorganisms, either via the food product (ingredient source) or via the packaging (product container and sealing component). The next two modules were focused on the sterilization step (again from the product or the packaging). The two following modules quantified the possible post-process re-contamination during intermediate storage (considering recontamination by air and biofilm formation for the product, only by air for the packaging). The last three modules were focused on the bottle filling and sealing operations in the filler cabinet (pre-filling, filling, sealing and storage modules). The "risk" of not complying with commercial sterility was defined as the rate of sterility failure and computed as the sum of product units contaminated by one or more bacteria, divided by the total number of product units produced for a specific line, product, packaging and factory. The risk was estimated for each bacterium of interest, i.e. for *C. botulinum*, *B. cereus* and *G. stearothermophilus* independently. It was computed at the end of module 9 (final risk) and also at the end of each process step.

2.2. Model inputs

The QMEA model had three types of inputs. The first one is directly link to management options. Indeed, the process settings are variables which enable control of the process. Their value can be changed to reduce the risk. For instance, the temperature of the UHT treatment is a thermal process setting. When building the QMEA model, they were set to a single value (average value in a baseline scenario representative of a generic factory line), i.e. they were deliberately considered as deterministic inputs. The second type of inputs corresponds to deterministic inputs which are not settings. It is impossible to associate any management option with these inputs. For instance, the density of raw materials is a variable required in the model, but it cannot be used to control and/or reduce the risk. The last type of inputs is the probabilistic inputs. They are set to a range of values with their associated probability of occurrence. Probabilistic inputs could reflect variability, uncertainty or both. The variability captures the biological diversity (Thompson, 2002), for example the diversity in *G. stearothermophilus* strains' heat resistance (D-value at 121 °C, D_{121}). In the model, variability was described mostly by Normal, Lognormal, Uniform or Pert distributions. The uncertainty captures the lack of knowledge (lack of data, lack of certainty of subject-matter experts of the domain), for example, the lack of knowledge to build precisely the distribution of *G. stearothermophilus* D_{121}. In the model, uncertainty was described by Uniform distributions. In this example, the input "D_{121}" had both variability and uncertainty dimensions which were introduced separately in the model as follows. The variability was described by a Pert distribution, Pert (5th; Most likely; 95th), truncated at 0, the uncertainty was described through three Uniform distributions: 5th ~ Uniform (0.725; 0.875), M. likely ~ Uniform (2.35; 2.45), 95th ~ Uniform (2.96; 3.04). The range of the Uniform distributions depends on the certainty with which the expert(s) gives the values (Guillier et al., 2013). In our study, the experts were specialists of aseptic UHT dairy product line with more than 20 years of work experience. In total, in the QMEA model, among the 128 probabilistic inputs, 31 had both variability and uncertainty dimensions and 97 probabilistic inputs had only one dimension (variability or uncertainty). In the case of the 31 inputs with both variability and uncertainty dimensions, if each input had variability described by a Pert distribution, itself described by three probabilistic distributions characterizing the uncertainty, there would have been 4 (1 for the variability + 3 for the uncertainty) probability distributions for each of the 31 inputs. As the variability dimension was not systematically described by a Pert, there was actually a total of 117 probability distributions: 31 for the variability + 86 for the uncertainty. The probabilistic inputs required to build the scenario presented in this study are provided in Table 1.

2.3. Second order Monte Carlo simulation

All calculations were made in Microsoft Excel using the @Risk 6.3.1 software (Palisade Corporation). The simulation process was performed using the Latin Hypercube sampling technique. The second order Monte Carlo simulation was performed as follows. First, a sample of 1000 values from each uncertainty distribution (86 in total) was generated and stored in a matrix (86 columns, 1000 rows). Next, for each realization of uncertainty (each row of the uncertainty matrix), a simulation was run using the "RiskSimtable" function of @Risk. For each simulation, 10,000 iterations were generated; this was done per bacterium (three bacteria). The seeds of each simulation were picked randomly. To avoid too much storage in Excel (costly in time and machine power), only the outputs relevant for the scenario of interest were collected. The results were compiled as follows: at the end of each simulation, the mean, 5th and 95th percentile values were stored in Excel. That provided information on the variability dimension. Then, using the outputs of the simulation run 1000 times (1000 realizations of uncertainty), an uncertainty interval was built around the mean, 5th and 95th percentile values. Obviously, the model output accuracy depended on the number of iterations; it was estimated to 2×10^{-8} (tested by running the model three times). Hereafter, if not mentioned otherwise, the risk is summarized per bacterium by its mean and 95th percentile values, both of them being characterized by their median and 95% uncertainty interval.