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► To cite this version:

Anne Laure Afchain, Frederic Carlin, Christophe Nguyen The, Isabelle Albert. Improving quantitative exposure assessment by considering genetic diversity of *B. cereus* in cooked, pasteurised and chilled foods. *International Journal of Food Microbiology*, 2008, 128 (1), pp.165-173. 10.1016/j.ijfoodmicro.2008.07.028 . hal-01263592

HAL Id: hal-01263592

<https://hal.science/hal-01263592>

Submitted on 31 May 2020

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1 **Improving quantitative exposure assessment by considering genetic**
2 **diversity of *B. cereus* in cooked, pasteurised and chilled foods**

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Abstract

The natural contamination of foods with a bacterial pathogen frequently consists of a mixture of strains with their own characteristics of survival, growth potential and virulence. Quantitative Microbial Risk Assessment (QMRA) must account for this genetic diversity to reflect the variability of the pathogen risk and to identify the genetic groups present at key stages of the food pathway.

To describe the transmission dynamics of a heterogeneous population of *B. cereus*, we developed an exposure model that covers a food processing chain from “farm to table”. The studied food was a cooked, pasteurized and chilled courgette purée used as an example of Refrigerated Processed Food of Extended Durability (REPFED). The *B. cereus* population consists of a continuum of genetic groups ranging from mesophilic and highly-heat resistant, to psychrotrophic and moderately-heat resistant ones. At each step in a processing chain comprising cooking, blending, mixing with ingredients providing a secondary contamination, pasteurisation and chilling for several weeks, the prevalence of contaminated units (batches or packages) and the spore load within the units were determined for each genetic group, as well as their proportion to the total *B. cereus* population in the units. The model predicted that all packages contain mesophilic groups just after partitioning. The addition of mesophilic strains by the ingredients during the process of the courgette purée was an important contribution. At the end of the domestic storage, the model predicted a dominance of the mesophilic groups, while only some psychrotrophic groups were present.

Keywords

Bacillus cereus, Bacterial diversity, Quantitative microbial risk assessment, Modelling, Food production chain, Minimal processing, Monte Carlo simulation.

38

39 1. Introduction

40 Most quantitative microbial risk assessments (QMRA) in foods performed over the
41 last twenty years were focussed on a single pathogen/food combination. However,
42 within many species, bacterial foodborne pathogenic bacteria generally show genetic
43 diversity, which may correspond to variable properties of resistance (to heat in
44 particular), of adaptation to low temperatures; and/or of virulence. For instance,
45 diarrhoeagenic *E. coli* are categorized into specific groups based on virulence
46 properties with some pathotypes, such as EHEC (and specifically *E. coli* O157:H7),
47 some of which being more resistant to acidity (Meng et al., 2007). Similarly, only
48 three out of the known serovars of *Listeria monocytogenes* account for more than 90%
49 of human and animal cases of listeriosis (Swaminathan et al., 2007).

50 *Bacillus cereus* is a common cause of foodborne gastro-enteritis in heat
51 processed, convenience foods and its broad diversity was recently acknowledged as a
52 source of difficulty for risk assessment in foods (EFSA, 2005). The genetic structure
53 of *Bacillus cereus* groups was recently resolved and the main features linked to
54 adaptation and to survival of the genetic groups were described (Guinebretière et al.,
55 2008). The growth domains of the *B. cereus* genetic groups range from nearly
56 thermophilic to psychrotrophic strains. The more thermophilic strains grow up to 55
57 °C or higher. The more psychrotrophic strains are able to grow at 5 °C and seem to be
58 less heat-resistant (Guinebretière et al., 2008). Carlin et al. (2006) also observed
59 different percentages in spore germination at chilled temperatures among the *B.*
60 *cereus* genetic groups. *B. cereus* is responsible of diarrhoea and emetic symptoms
61 (Granum 2007). The virulence of each strain is not clearly demonstrated, but some
62 genetic groups are likely or potentially more virulent (genetic proximity with *Bacillus*

anthracis, for instance) (Guinebretière et al., 2008) and some of the psychrotrophic strains seem to have a low ability to cause gastro-enteritis (Choma et al., 2000; Carlin et al., 2006; Guinebretière et al., 2008).

The studied food was a cooked, pasteurized and chilled vegetable courgette purée, manufactured and stored in a complex food pathway, representative of that of many other cooked chilled foods. Extensive data on microbial flows in these courgette purées, including prevalence and concentrations of *B. cereus* at different processing steps, were available in recently published papers (Guinebretière et al., 2003; Guinebretière and Nguyen-The, 2003). Previous microbial risk assessments on courgette purées (Nauta, 2001; Nauta et al., 2003; Malakar et al., 2004) assumed that each product was contaminated by one type of strain, either mesophilic or psychrotrophic. The final product was then either contaminated, or not, by this type. In Nauta (2001), the model was run for a single strain, neglecting the prevalence of other strains. However, real foods can be contaminated by a mixture of various genetic groups and, to assess the proportions of the various groups at each step of the chain, it is interesting to consider all the groups in the same model run.

Using the same quantitative risk assessment scheme as Nauta et al. (2003), we studied the fate of a *B. cereus* population modelled as a complex mixture of strains belonging to the genetic groups defined by Guinebretière et al. (2008). The objective of this work was to develop an exposure model able to predict the proportion of each *B. cereus* genetic group at each step of processing and storage, as well as the number of contaminated packages and the mean concentration in *B. cereus* per genetic group. The model was built using a classical approach of Monte Carlo simulation, with the whole bacterial population consisting in a mixture of groups, each having relatively variable genetic characteristics.

2. Materials and methods

An exposure model before consumption was implemented following the Modular Process Risk Model (MPRM) methodology (Nauta, 2002), i.e the food pathway was split up into smaller stages. Each stage was identified as one basic process or consumer operation associated with the pathogen transmission. It allowed the assessment of the final contamination of a food from the input variables (parameters) that acted on this contamination. Numerical simulation methods by Monte-Carlo were then used in order to compute the distribution of the final contamination from variability and/or uncertainty distributions attributed to each of these model parameters. Thus, these distributions reflected variability and uncertainty of characteristics within the genetic groups that they represented.

2.1 Data on the initial contamination in the vegetable and its ingredients

The initial contamination levels of each *B. cereus* genetic group came from observed data published in former studies on the contamination with aerobic spore-forming bacteria in a courgette purée processing line (Guinebretière et al., 2003; Guinebretière & Nguyen-the, 2003). We used the genetic typing of *B. cereus* strains isolated from the product at various processing steps (Guinebretière & Nguyen-the, 2003) to retrospectively attribute each strain to one of the seven genetic groups numbered I, II, III, IV, V, VI and VII defined by Guinebretière et al. (2008) and which main characteristics are summarized in Table 1. We did not consider group I, because none of the strains within this group was isolated from foods (Guinebretière et al., 2008). Groups II and VI are psychrotrophic, groups III, IV and VII are mesophilic and group V has an intermediate behaviour. Psychrotrophic groups II and VI prevailed in the raw courgette, whereas mesophilic groups III and IV prevailed in the ingredients (milk proteins and starch). In the other ingredients (UHT dairy cream and salt), no *B.*

cereus was detected (Guinebretière & Nguyen-the, 2003). Some strains of groups II, III, IV, V and VII were associated with foodborne illnesses, but none of group VI (Guinebretière et al., 2008).

2.2 The food pathway

Commercial cooked, pasteurized and chilled courgette purées considered in this work were manufactured by a factory located in Normandy, France. Figure 1 gives the flow diagram of the food processing chain from “farm to table”. Table 2 gives the constant values or the variability distributions of time-temperature combinations (t, T) for each stage. These were estimated from the data recorded by the company, expert’s opinion of the company staff or literature data.

During processing, fresh courgettes were firstly trimmed and peeled, washed, then steam cooked for a mean equivalent time at 90°C of 161.4 min and blended for 15 min at a temperature of 80 °C (Table 2). The mixing process included 300 kg of cooked and blended courgettes and 44 kg ingredients (29 kg of UHT dairy cream and salt, 10 kg of starch, and 5 kg of milk proteins) and produced a courgette purée batch. Starch and milk proteins were a second source of bacterial contamination (Guinebretière et al., 2003).

Then, the batch was split up into 860 packages of courgette purée. Partitioning means portioning of a 344 kg batch into 860 packages of 400 g each. The random distribution of the spore numbers within the packages after partitioning (Nauta, 2005) was modelled with a Multinomial distribution, more valid than the Poisson approximation for low numbers of spores, i.e. those of the undetected genetic groups by enrichment. Mixing and partitioning of the courgette purée were in practice very brief events and were consequently assumed to be instantaneous.

The packaged courgette purées were then pasteurised in a water bath at the reference temperature of 90 °C for a mean equivalent time estimated to be 4 min. A time interval between partitioning and pasteurisation, modelled by a Gamma distribution with a mean duration of 55.5 min and a mean temperature of 46 °C, generally occurred during processing.

Before leaving the factory, the packages were stored at a refrigeration temperature of 4 °C for 4 days on average in the company warehouse, and then were sent to the retail establishments by refrigerated road transport. During retail, they were stored in refrigerated cabinets till purchase by consumers, home transportation and home storage in refrigerators before consumption (Table 2).

2.3 Microbial basic processes

We attributed to each stage of the food pathway one or two microbial basic biological processes (Table 3). We assumed that cooking, blending of a still hot cooked courgettes and pasteurisation caused *B. cereus* inactivation; and that chill chain in the processing plant, at the retail shop, in transports and in the domestic refrigerator allowed *B. cereus* growth.

2.3.1 Thermal inactivation model

The thermal inactivation model used (Mafart et al., 2002; van Boekel, 2002) applied to each genetic group:

$$\log_{10} \left(\frac{N_t^s}{N_0^s} \right) = - \left(\frac{t}{\delta'_s} \right)^{p_s} \quad (1)$$

where

- t (min) is the duration of the thermal process,

- 160 - N_t^s (CFU/g) is the modelled bacterial concentration of genetic group s after a
161 thermal process for time t ,
- 162 - N_0^s (CFU/g) is the initial bacterial concentration of genetic group s at the start of
163 thermal process,
- 164 - p_s is the shape parameter of the Weibull distribution for genetic group s ,
- 165 - δ'_s (min) is the time to the first decimal reduction time of the spore population for
166 genetic group s under an iso-thermal regime at T (°C), computed from the following
167 relation (Couvert et al., 2005):

$$\delta'_s = \delta_s^r 10^{(T_r - T)/z} \quad (2)$$

- 170 where
- 171 - T (°C) is the temperature of the product,
- 172 - T_r (°C) is the reference temperature, fixed at 90 °C,
- 173 - δ_s^r (min) is the calculated δ -value corresponding to $T=T_r$ for genetic group s ,
- 174 - z (°C) is the increase in temperature resulting in a 10-fold reduction in the time to
175 first decimal reduction δ .

176 Time-temperature profiles of raw vegetable cooking and pasteurisation of the
177 courgette purées recorded by the company were converted into an equivalent heat
178 treatment of time $t=t_{eq}$ at the target temperature $T_r=90$ °C using Equation (3)
179 (Fernandez and Peck, 1997), in order to account for the product temperature
180 variability observed during these two thermal processes. Equation (3) is as follows:

$$t_{eq} = \sum_{k=1}^n (t_k - t_{k-1}) 10^{\frac{1}{z} \left(\frac{T_k + T_{k-1}}{2} - 90 \right)} \quad (3)$$

- 182 where

- $t_k - t_{k-1}$ (min) is the time interval between two temperature measurements,
- T_k (°C) is the k -th observed temperature in sample ($T_0, \dots, T_n$) constituted by $n+1$ temperatures $T_0, \dots, T_n$, where T_n is measured at the end of the thermal process.
- z (°C) is the increase in temperature resulting in a 10-fold reduction in the time to first decimal reduction δ , as previously described.

2.3.2 Spore germination

We assumed that uncooked courgettes, starch and milk proteins were initially contaminated with spores only (Table 4), and we assumed that spore germination was relevant for the time interval between partitioning and pasteurisation (Table 2), and at the beginning of chill chain, because *B. cereus* spores germinate and grow in courgette purée (Choma et al., 2000), and because temperatures monitored during these processes were compatible with spore germination. Reduction in spore numbers during cooking, blending and pasteurisation was described by a heat inactivation model (Eq. 1, 2 and 3).

Spores might germinate during the time interval between partitioning and pasteurisation, as the recorded temperature decreased enough. Germinated spores were then assumed to be killed during pasteurisation, as the temperature during this process was substantially higher than 70 °C for 15 min. In other words, populations of the genetic groups with the highest germination rate will be the most reduced by pasteurisation. Within the range of the considered temperatures, the germination was assumed to occur below 45°C for spores of groups III, IV, V and VII, below 42°C for spores of group II, and only below 37°C for spores of group VI. When germination occurs, it was also assumed to be instantaneous, and that 90 % of the spores of each group germinate (Johnson et al., 1983; Nguyen-the and Carlin, unpublished data). After a pasteurisation equivalent to 4 min at 90 °C, which eliminates vegetative cells,

208 *B. cereus* populations consisted *de facto* of spores only. During subsequent storage
209 periods, all spores were assumed to germinate, and growth depended only on the
210 growth parameters of each genetic group.

211 2.3.3 Growth model

212 The primary growth model used was the logistic model with delay (Rosso et al., 1995)
213 applied to each genetic group, as follows:

$$214 \quad N_t^S = \begin{cases} \frac{N_{\max}}{1 + \left(\frac{N_{\max}}{N_0^S} - 1 \right) e^{-\mu_{\max}^S (t - \lambda)}} & \text{if } t \geq \lambda \\ N_0^S & \text{if } t < \lambda \end{cases}$$

215 (4)

216 where

- 217 - t (h) is the duration of the considered stage in the food pathway,
- 218 - N_t^S (CFU/g) is the modelled bacterial concentration of genetic group s after a
219 growth period for time t ,
- 220 - N_0^S (CFU/g) is the modelled initial bacterial concentration of genetic group s at the
221 beginning of the considered stage,
- 222 - $N_{\max}$ (CFU/g) is the modelled maximum bacterial concentration in the product,
223 assumed to be the same for the six genetic groups,
- 224 - $\mu_{\max}^S$ (h^{-1}) is the maximum growth rate for genetic group s ,
- 225 - λ is the lag time and equal to 0 in this work as no proper value was available for
226 any of the *B. cereus* genetic groups. In addition, this is a fail-safe assumption.

227

228 The secondary model used was a CTMI (CTMI: cardinal temperature model with
229 inflection point) model (Rosso et al., 1993) characterized for each genetic group:

$$230 \quad \mu_{\max}^s(T) = \begin{cases} \frac{\mu_{opt} (T - T_{\max}^s)(T - T_{\min}^s)^2}{(T_{opt}^s - T_{\min}^s)[(T_{opt}^s - T_{\min}^s)(T - T_{opt}^s) - (T_{opt}^s - T_{\max}^s)(T_{opt}^s + T_{\min}^s - 2T)]} & T \in [T_{\min}^s; T_{\max}^s] \\ 0 & T \notin [T_{\min}^s; T_{\max}^s] \end{cases}$$

231 (5) where

- 232 - μ_{opt} (day^{-1}) is the optimal growth rate in the product,
- 233 - T ($^{\circ}\text{C}$) is the core temperature of the product,
- 234 - $T_{\min}^s$ ($^{\circ}\text{C}$) is the minimal cardinal temperature for genetic group s ,
- 235 - T_{opt}^s ($^{\circ}\text{C}$) is the optimal cardinal temperature for genetic group s ,
- 236 - $T_{\max}^s$ ($^{\circ}\text{C}$) is the maximal cardinal temperature for genetic group s .

237 The whole *B. cereus* population, considered as a mixture of genetic groups II-
238 VII, cannot exceed $N_{\max}$ i.e. no genetic group is able to continue its growth once the
239 whole population of *B. cereus* reaches $N_{\max}$. This proposition can be formalized in the
240 following expression:

$$241 \quad N_t^s = \begin{cases} N_t^s & \text{if } \sum_{k=1}^n N_t^k \leq N_{\max} \\ N_{\max} \frac{N_t^s}{\sum_{k=1}^n N_t^k} & \text{otherwise} \end{cases} \quad (6)$$

242 where

- 243 - $N_{\max}$ (CFU/g) is the maximum bacterial concentration in the product, as previously
- 244 defined,

- 245 - N_t^s (CFU/g) is the bacterial concentration of genetic group s after a growth period
- 246 for time t when satisfying or not the constraint of the maximal concentration in the
- 247 product,
- 248 - N_t^s (CFU/g) is the bacterial concentration of genetic group s after a growth period
- 249 for time t , as previously defined in Equation (4) (i.e. the whole bacterial population
- 250 did not exceed $N_{\max}$),
- 251 - n is the number of genetic groups.

252 2.4 Monte-Carlo simulation

253 The stochastic exposure model before consumption was elaborated by Monte Carlo

254 simulation using R software (R Development core Team, 2004). The parameters of

255 the exposure model were the initial contamination level for the six genetic groups (II

256 to VII), the time and temperature combinations for each stage of the food pathway,

257 the thermal inactivation parameters, the germination rate and the bacterial growth

258 parameters, and they were represented by defined values, variability distributions,

259 and/or uncertainty distributions (Frey & Burmaster, 1999). For each simulation, 2000

260 iterations, representing 2000 independent industrial batches were run in the exposure

261 model before consumption, to consider the variability of the initial contamination and

262 the process variability and/or uncertainty within each genetic group. At each iteration

263 (or batch), the six (II to VII) groups of the *B. cereus* population, with various genetic

264 characteristics and concentrations were followed along the food pathway. In the

265 present simulation model, each *B. cereus* genetic group underwent inactivation,

266 germination and growth with specific parameters independently of each other. After

267 mixing, the *B. cereus* population from the batch was pooled with the *B. cereus*

268 population from the ingredients to form a new population. After partitioning, this

population was distributed into the packages, which subsequently followed their own pathway in the chain (one time and temperature history at each stage). After partitioning of one batch into 860 packages, only 100 packages of contaminated vegetable purées were followed until the home refrigeration for reasons of computer memory capacity. Partitioning into 100 packages gave the same distribution of the genetic groups among packages as partitioning into 860 packages (data not shown).

The initial contamination levels for each genetic group were modelled by Beta-Pert distributions (Vose, 2000), each sampled by batch to take the variability of the initial contamination into account. *B. cereus* initial concentrations in courgette and courgette purée ingredients were too low to enumerate using direct plating and were detected after enrichment only. For the groups detected by enrichment, the distribution of concentrations was contained in the interval, which lower and upper bounds were respectively the limit of detection of the enrichment procedure and the limit of enumeration by direct plating. For the genetic groups undetected by enrichment, we assumed that they were present, but in concentrations lower than the limit of detection, which represents a fail-safe assumption. The distribution of *B. cereus* concentrations had the same width as that of the genetic groups detected by enrichment. Their parameters were shown in Table 4. The most likely value of Beta-Pert distributions corresponded to the middle of the interval on which concentrations were distributed.

Table 5 shows the heat-resistance parameters of the *B. cereus* genetic groups estimated from a collection of strains representative of groups II ($n = 7$), III ($n = 35$), IV ($n = 27$), V ($n = 2$), VI ($n = 11$) and VII ($n = 2$). Parameter estimation and strain typing were performed in previous works (Carlin et al., 2006; Guinebretière et al., 2008). In our exposure model, a Beta-Pert distribution was used for p_s (see Eq. 1) and

δ_s^r (see Eq. 2). For some heat resistant strains (group III), curve fitting did not allow a proper determination of δ -values. For those strains, a δ -value of 180 min was chosen by default. The parameters of the distribution of z values (see Eq. 2 and Table 5), assumed to be independent of the genetic groups, were estimated from 94 literature data (Gilbert et al., 1974; Bradshaw et al., 1975; Johnson et al., 1983; Rajkowski & Mikolajcik, 1987; Picoche et al., 1993; Mazas et al., 1995; ICSMF, 1996; Faille et al., 1997; Gonzalez et al., 1997; Gaillard et al., 1998 (a), 1998 (b); Fernandez et al., 1999; Gonzalez et al 1999; Mazas et al., 1999; Mafart et al., 2002) and followed a log-normal distribution truncated on a temperature interval. The median was 8.3 °C. For cooking and pasteurisation, an exponential distribution was fitted to the equivalent times t_{eq} computed from Equation (3) on 5 and 40 time-temperature profiles recorded by the company, respectively. In this computation, an uncertainty on z value (see Eq. 2 and Table 5) was inserted.

We assumed that cardinal temperatures T_{min}^s , T_{opt}^s and T_{max}^s (see Eq. 5) follow normal distributions. Their mean values are shown in Table 6. Their standard deviation was fixed to 1° C for all *B. cereus* genetic groups II to VII, in absence of published data. For *Listeria monocytogenes* for instance, standard deviation for T_{min} was 0.7 °C when estimated for a large set of culture conditions and strains (Pouillot et al., 2003). Table 6 also gives the variability distributions of μ_{opt} (see Eq. 5) and N_{max} (see Eq. 4-6) in courgette purée, which values were assumed to be independent of the genetic groups. The μ_{opt} -value was derived from previous μ_{max} (see Eq. 5) data in a courgette broth (Choma et al., 2000). In that study, the inoculum was made of strains able to grow at 6.5°C, which suggests the strains were of genetic groups II, V or VI that contain psychrotrophic *B. cereus*. The variability distribution on μ_{opt} was

assumed to be a Beta-Pert distribution, with parameters estimated to 0.58 h^{-1} , 0.70 h^{-1} , 1.04 h^{-1} , respectively (Table 6). For $\log N_{\max}$, Choma et al. (2000) studied a broad range of products and reported maximal numbers of *B. cereus* at the end of refrigeration storage reaching $8.5 \pm 0.6 \log \text{ CFU/g}$. Thus, we assumed that this last parameter followed a normal distribution with a mean of $8.5 \log \text{ CFU/g}$ and a standard deviation of $0.6 \log \text{ CFU/g}$ (Table 6).

Only packs of purée units containing *B. cereus* represent a hazard for consumers. The relevant information to assess consumer exposure is therefore the concentration of *B. cereus* in positive units, combined with the prevalence of positive units, and addressing a null risk to negative units. At main key stages of the food pathway (initial contamination of 300 kg batches of raw courgettes, after cooking of 300 kg batches, after mixing with ingredients and partitioning into 400 g packages, after pasteurisation of 400 g packages and at the end of home storage in refrigerators), the prevalences of packages containing at least one *B. cereus* spore, whatever the genetic group, were computed, and the bacterial load ($\log \text{ CFU/g}$) of the contaminated units (batch or package) per genetic group was calculated, taking into account units containing at least one CFU of this group. The proportions of each *B. cereus* genetic group to the whole *B. cereus* population in the batches or packages were also calculated, considering all units.

3. Results

Before cooking, all genetic groups were predicted to be present in any unprocessed vegetable batches (Table 7), and the two psychrotrophic groups (II and VI) prevailed (Fig. 2A), as shown by Guinebretière and Nguyen-the (2003). After cooking, any genetic group was present in at least 30 % of batches (Table 7). Figure 3 and Table 8 present the changes of respectively *B. cereus* mean concentrations in positive units

(batch or packages) and also the corresponding 95% confidence intervals (CI) per genetic group, reflecting the uncertainty assumed in the model. There was a ranking of the genetic groups as a function of their resistance to cooking (Fig. 3). The mesophilic group VII was the most resistant of all groups, and among the psychrotrophic groups, group II was the most resistant, whereas group VI was the most sensitive to heat. This resulted in a major decrease in the number of the psychrotrophic group VI after cooking in positive units. However, their prevalence (47%) in units after cooking was similar to that of the more heat resistance group IV (49%), because group VI was more abundant in the uncooked courgette (Table 7). The upper bounds of CI were relatively unchanged by cooking (Table 8). The lower bound of CI corresponded to one spore in a 300 kg batch. Among the psychrotrophic groups, group II prevailed, because of heat-resistance higher than that of group VI (Fig. 2B). The proportions of groups III, IV, and VII, rare in uncooked vegetables (Fig. 2A), increased after cooking (Fig. 2B) likely because of their high resistance to heat and the corresponding decrease from 48.3% to 13.1% of the proportion of the more heat sensitive group VI.

The addition of ingredients to the cooked courgette (Fig. 4A) further increased the mean proportions of the mesophilic groups (groups III and IV) compared to the psychrotrophic groups (groups II and VI), as experimentally observed by Guinebretière and Nguyen-the (2003). In conclusion, cooking and addition of ingredients resulted in a marked change in the *B. cereus* population, from psychrotrophic to mesophilic groups (Fig. 2A and Fig. 4A).

After partitioning, all the 400-g packages were contaminated by group IV; group III was present in about 81% of the packages and group II in 33 % (Table 7). The prevalence of groups V, VI and VII in the courgette purées packages after

partitioning was much lower (below 10%) than that of the other groups. This was likely due to a low concentration of these groups in the batch before partitioning. In particular, because of partitioning, group VI, the most able to multiply during chill chain, was undetectable from the majority of packages.

Pasteurisation following germination achieved a strong, but not total, reduction of spores, and modified the proportions of the genetic groups among the packages (Table 7). The prevalence of positive packages decreased for all groups. Groups V, VI and VII reached prevalences below 1%. However, the concentrations per positive package were already low before the pasteurisation process (Fig. 3 and Table 8), close to the limit of 1 CFU per package (i.e. $-2.6 \log \text{CFU/g}$). The predicted mean concentration of the genetic groups in contaminated packages remained nearly unchanged (Fig. 3). Compared to cooking, pasteurisation slightly affected the proportion of the genetic groups (Fig. 4B) and group IV was still the most represented in the packages after pasteurisation (about 75% of the whole *B. cereus* population). No additional inactivation of *B. cereus* was done on the sealed packages: the prevalence of the genetic groups (Table 7) remained consequently unchanged in steps following pasteurisation.

At the end of home refrigeration, very few of packages (0.07%) had predicted cell concentrations equal to $N_{\max}$ (Eq. 6). During the simulated chill chain, mean concentrations (Fig. 3) of *B. cereus* genetic groups II and VI increased by approximately 2.1 and 3.8 $\log \text{CFU/g}$, whereas groups V and IV increased much less, and groups III and VII showed almost no growth on average during the simulated shelf-life. At the end of home storage in refrigerators, group II and VI had the highest mean concentrations per positive package (approximately by 0.2 and 1.2 $\log \text{CFU/g}$, respectively) (Fig. 3). These groups were present in only 19% and 0.1% of packages

respectively (Table 7). The simulation model was consistent with observations from the company processing the vegetable purees, i.e. that packages rarely contained concentrations of *B. cereus* detectable by direct plating (threshold of 2 log CFU/g) at the end of shelf-life when storage between 4 and 8°C for 21 or 28 days was simulated (Nguyen-the, unpublished data). The model predicted that, at the end of home storage in refrigerators, only 2.5% of the packages contaminated by group II exceeded a bacterial concentration of 6.7 log CFU/g (Table 8). As shown by Figure 5, these high concentrations were typically associated with low concentrations of genetic group III (a mesophilic group). However, some high concentrations of genetic group II (a psychrotrophic group) were associated with medium or high concentrations of genetic group III (Fig. 5). From Figure 4C, a slight increase in the mean proportion of the psychrotrophic groups was observed at the end of the home refrigeration, due to their ability to grow at lower temperatures than the other groups. In particular, group II represented on average 16.8% of the *B. cereus* population per package. Group V, which heat-resistance and ability to grow at low temperature was between those of psychrotrophic group II and mesophilic group IV, could have an intermediate behaviour. However, in our model, this group was rare over all the processing chain, presumably because of a low prevalence in raw material and ingredients.

4. Discussion

Considering a mixture of pathogenic *B. cereus* strains along a food pathway in a single exposure model gave a representation of the qualitative and quantitative genetic diversity within the whole bacterial population. Particularly, our simulation suggested that, at the end of home refrigeration (and therefore at the time of consumption), *B. cereus* may be present in all the packages of courgette purée, but also that all *B. cereus* genetic groups were not present in all packages. The proportion of each genetic

group relatively to the whole *B. cereus* population was strongly influenced by processing, storage and contamination routes. By integrating the various components of a bacterial population in a single model, instead of running various models independently for each component, we were able to simulate the prevalence of the genetic groups and the composition of the bacterial population (proportion between the different groups in the whole *B. cereus* population) at any stage of the food pathway. The mean proportion of all genetic groups cannot be interpreted independently of bacterial concentration in contaminated packages and of prevalence of each genetic group. According to the exposure model, the most psychrotrophic group VI was eliminated from most of the packages because of low heat resistance, but it reached the highest predicted *B. cereus* populations in some packages (indicated by the upper bounds of confidence intervals). In contrast, the prevailing groups were the mesophilic ones, which better resisted the heat treatments, but with a much lower growth potential during refrigeration storage. Their concentrations rarely exceeded 10^3 CFU/g, which usually is not a concern for food safety (Granum, 2007). A proper risk assessment should also include the virulence properties of each genetic group. Most of the strains related to cases or outbreaks of *B. cereus* foodborne poisoning belong to groups III and IV (Guinebretière et al. 2008). Considering psychrotrophic groups, Stenfors et al. (2002) observed that *B. weihenstephanensis*, which belongs to *B. cereus* group VI (Guinebretière et al., 2008), was poorly cytotoxic on Vero cells compared to other *B. cereus*. Similarly, Choma et al. (2000) found the most psychrotrophic strains of *B. cereus* the least toxic on Caco2 cells. According to Svensson et al. (2007), most of the strains isolated from refrigerated pasteurized milk were *B. weihenstephanensis* (i.e. *B. cereus* group VI) and produced very low amount of enterotoxins. These works strongly suggest that *B. cereus* group VI is less prone to

cause diarrhoea than other groups. In our work, *B. cereus* group II was predicted to prevail over genetic group VI after refrigerated storage, presumably because its spores were more heat resistant and the heat treatments applied in our process were more severe than for milk pasteurisation. The virulence of *B. cereus* group II is not characterized, but as some strains implicated in foodborne poisoning belong to group II (Guinebretière et al., 2008), it is presumably more virulent than group VI, which does not contain any of the known strains associated with cases or outbreaks of foodborne poisonings (Guinebretière et al., 2008). Therefore, the risk of *B. cereus* foodborne poisoning linked to refrigerated heat processed foods will presumably be associated with the presence and growth of this group of psychrotrophic (group II) *B. cereus* in the food at consumption. Finally, a major perspective of the present work would be to determine what makes the highest contribution to the risk for the consumer, between a low *B. cereus* prevalence in processed foods and a high growth potential (as those of the psychrotrophic genetic groups) on one side, and on the other side a high prevalence with a lower growth potential (as those of the mesophilic groups). In conclusion, a simulation tool was designed to predict the fate of the various components of a complex *B. cereus* population of pathogens during food processing. The exposure model will facilitate the assessment of the risk due to different genetic groups of *B. cereus* when new food processes are evaluated.

Acknowledgements

This work was supported by a grant from the Agence Nationale de la Recherche (ANR) (France) as part of an ANR-05-PNRA-013 *B. cereus* contract. Thanks are due to Sandrine Auger, Alexei Sorokine (INRA Jouy-en-Josas, France), and to the Sym'Previous consortium ([www. Symprevious.net](http://www.Symprevious.net)) for kind communication of cardinal growth temperatures of some of the genetic *B. cereus* groups.

References

- Auger S, Galleron N, Bidnenko E, Ehrlich SD, Lapidus A, Sorokin A., 2008. The genetically remote pathogenic strain NVH391-98 of the *Bacillus cereus* group represents the cluster of thermophilic strains. Applied and Environmental Microbiology 74, 1276-1280.
- Bradshaw, J. G., Peeler, J. T., Twedt, R. M., 1975. Heat resistance of ileal loop reactive *Bacillus cereus* strains isolated from commercially canned food. Applied Microbiology 30, 943-945.
- Carlin, F., Fricker, M., Pielaat, A.M., Heisterkamp, S., Shaheen, R., Salonen, M.S., Svensson, B., Nguyen-the, C., Ehling-Schulz, M., 2006. Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics within the *Bacillus cereus* group. International Journal of Food Microbiology 109, 132-138.
- Choma, C., Guinebrière, M.H., Carlin, F., Schmitt, P., Velge, P., Granum, P.E., Nguyen-the, C., 2000. Prevalence, characterization and growth of *Bacillus cereus* in commercial cooked chilled foods containing vegetables. Journal of Applied Microbiology 88, 617-625.
- Couvert, O., Gaillard, S., Savy, N., Mafart, P., Leguérinel, I., 2005. Survival curves of heated bacterial spores: effect of environmental factors on Weibull parameters. International Journal of Food Microbiology 101, 73-81.
- EFSA, 2005. Opinion of the scientific panel on biological hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. The EFSA Journal 175, 1-48.
- Faille, C., Leuret, V., Gavini, F., Maingonnat, J. F., 1997. Injury and lethality of heat treatment of *Bacillus cereus* spores suspended in buffer and in poultry meat. Journal of Food Protection 60, 544-547.

- 492 Fernandez, P. S., Peck, M. W., 1997. Predictive model describing the effect of
493 prolonged heating at 70 to 80°C and incubation at refrigeration temperatures on
494 growth and toxigenesis by nonproteolytic *Clostridium botulinum*. Journal of Food
495 Protection 60, 1064-1071.
- 496 Fernandez, A., Ocio, M. J., Fernandez, P. S., Rodrigo, M., Martinez, A., 1999.
497 Application of nonlinear regression analysis to the estimation of kinetic parameters
498 for two enterotoxigenic strains of *Bacillus cereus* spores. Food Microbiology 16,
499 607-613.
- 500 Frey, H.C., Burmaster, D.E., 1999. Methods for characterizing variability and
501 uncertainty: Comparison of bootstrap simulation and likelihood-based approaches.
502 Risk Analysis 19, 109-130.
- 503 Gaillard, S., Leguerinel, I., Mafart, P., 1998 (a). Modelling combined effects of
504 temperature and pH on the heat resistance of spores of *Bacillus cereus*. Food
505 Microbiology 15, 625-630.
- 506 Gaillard, S., Leguerinel, I., Mafart, P., 1998 (b). Model for combined effects of
507 temperature, pH and water activity on thermal inactivation of *Bacillus cereus*
508 spores. Journal of Food Science 63, 887-889.
- 509 Gilbert, R. J., Stringer, M. F., Peace, T. C., 1974. The survival and growth of *Bacillus*
510 *cereus* in boiled and fried rice in relation to outbreaks of food poisoning. Journal of
511 Hygiene, Cambridge 73, 433-444.
- 512 Gonzalez, I., Lopez, M., Mazas, M., Gonzalez, J., Bernardo, A., 1997. Thermal
513 resistance of *Bacillus cereus* spores as affected by additives in the recovery
514 medium. Journal of Food Safety 17, 1-12.

- 515 Gonzalez, I., Lopez, M., Martinez, S., Bernardo, A., Gonzalez, J., 1999. Thermal
516 inactivation of *Bacillus cereus* spores formed at different temperatures.
517 International Journal of Food Microbiology 51, 81-84.
- 518 Granum, P. E.. 2007. *Bacillus cereus*, In: Doyle, M. P., Beuchat, L. R. (Ed.), Food
519 Microbiology: Fundamentals and Frontiers, 3rd ed. ASM Press, Washington D.C.,
520 pp. 445-456.
- 521 Guinebretière, M.H., Girardin, H., Dargaignaratz, C., Carlin, F., Nguyen-the, C.,
522 2003. Contamination flows of *Bacillus cereus* and spores-forming aerobic bacteria
523 in a cooked, pasteurized and chilled courgette purée processing line. International
524 Journal of Food Microbiology 82, 223-232.
- 525 Guinebretière, M.H., Nguyen-the, C., 2003. Sources of *Bacillus cereus* contamination
526 in a pasteurized courgette purée processing line, differentiated by two PCR-based
527 methods. FEMS Microbiology Ecology 43, 207-215.
- 528 Guinebretière, M.H., Thompson, F.L., Sorokin, A., Normand, P., Dawyndt, P.,
529 Ehling-Schultz, M., Svensson, B., Sanchis, V., Nguyen-the, C., Heyndrickx, M.,
530 De Vos P., 2008. Ecological diversification in the *Bacillus cereus* Group.
531 Environmental Microbiology 10, 851-865.
- 532 ICMSF, 1996. *Bacillus cereus*. In: Roberts, T. A., Baird-Parker, A. C., Tompkin, R.
533 B. (Ed.), Micro-organisms in foods. 5. Microbiological specifications of food
534 pathogens. Blackie Academic and Professional, London, pp. 20-35.
- 535 Johnson, K. M., Nelson, C. L., Busta, F. F., 1983. Influence of temperature on
536 germination and growth of spores of emetic and diarrheal strains of *Bacillus*
537 *cereus* in a broth medium and in rice. Journal of Food Science 48, 286-287.

- 538 Mafart, P., Couvert, O., Gaillard, S., Leguerinel, I., 2002. On calculating sterility in
539 thermal preservation methods: application of the Weibull frequency distribution
540 model. International Journal of Food Microbiology 72, 107-113.
- 541 Malakar, P.K., Barker, G.C., Michael, W. P., 2004. Modeling the prevalence of
542 *Bacillus cereus* spores during the production of a cooked chilled vegetable product.
543 Journal of Food Protection 67, 939-946.
- 544 Mazas, M., Gonzalez, I., Lopez, M., Gonzalez, J., Martin Sarmiento, R., 1995. Effects
545 of sporulation media and strain on thermal resistance of *Bacillus cereus* spores.
546 International Journal of Food Science and Technology 30, 71-78.
- 547 Mazas, M., Lopez, M., Martinez, S., Bernardo, A., Martini, R., 1999. Heat resistance
548 of *Bacillus cereus* spores: Effects of milk constituents and stabilizing additives.
549 Journal of Food Protection 62, 410-413.
- 550 Meng, J., Doyle, M. P., Zhao, T., Zhao, S., 2007. Enterohemorrhagic *Escherichia coli*.
551 In: Doyle, M. P., Beuchat, L. R. (Ed.), Food Microbiology: Fundamentals and
552 Frontiers, 3rd ed. ASM Press, Washington D.C., pp. 249-269.
- 553 Nauta, M.J., 2001. A modular process risk model structure for quantitative
554 microbiological risk assessment and its application in an exposure assessment of
555 *Bacillus cereus* in a REPFED. RIVM report, Bilthoven, 100pp.
- 556 Nauta, M. J., 2002. Modelling bacterial growth in quantitative microbiological risk
557 assessment: is it possible? International Journal of Food Microbiology 73(2-3),
558 297-304.
- 559 Nauta, M.J., Litman, S., Barker, G.C., Carlin F., 2003. A retail and consumer phase
560 model for exposure assessment of *Bacillus cereus*. International Journal of Food
561 Microbiology 83, 205-218.

- 562 Nauta, M.J., 2005. Microbiological risk assessment models for partitioning and
563 mixing during food handling. International Journal of Food Microbiology 100,
564 311-322.
- 565 Picoche, B., Denis, C., Pichon, P., 1993. Comportement des spores de *Bacillus cereus*
566 dans les légumes cuits sous vide. Industries Alimentaires et Agricoles 110 (6), 454-
567 459.
- 568 Pouillot, R., Albert, I., Cornu, M., Denis, J. B., 2003. Estimation of uncertainty and
569 variability in bacterial growth using Bayesian inference. Application to
570 *Listeria monocytogenes*. International Journal of Food Microbiology 81, 87-
571 104.
- 572 Rajkowski, K. T., Mikolajcik, E. M., 1987. Characteristics of selected strains of
573 *Bacillus cereus*. Journal of Food Protection 50, 199-205.
- 574 Rosso, L., Lobry, J.R., Flandrois, J.P., 1993. An unexpected correlation between
575 cardinal temperature of microbial growth highlighted by a new model. Journal of
576 Theoretical biology 162, 447-463.
- 577 Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient model to
578 describe the combined effects of temperature and pH on microbial growth. Applied
579 and Environmental Microbiology 61, 610-616.
- 580 R Devpt core Team, 2004. R: A language and environment for statistical computing.
581 <http://www.R-project.org>.
- 582 Stenfors, L. P. R., Mayr, S., Scherer, Granum, P. E., 2002. Pathogenic potential of
583 fifty *Bacillus weihenstephanensis* strains. FEMS Microbiology Letters 215, 47-51.
- 584 Svensson, B., Monthan, A., Guinebretière, M.H., Nguyen-the, C., Christiansson, A.,
585 2007. Toxin production potential and the detection of toxin genes among strains of

586 the *Bacillus cereus* group isolated along the dairy production chain. International
587 Dairy Journal 17, 1201-1208.

588 Swaminathan, B., Cabanes, D., Zhang & W., Cossart, P., 2007. *Listeria*
589 *monocytogenes*. In: Doyle, M. P., Beuchat, L. R. (Ed.), Food Microbiology:
590 Fundamentals and Frontiers, 3rd ed. ASM Press, Washington D.C., pp. 457-491.

591 van Boekel, M.A.J.S., 2002. On the use of the Weibull model to describe thermal
592 inactivation of microbial vegetative cells. International Journal of Food
593 Microbiology 74, 139-159.

594 Vose, D., 2000. Risk analysis: a quantitative guide. John Wiley & Sons, Ltd (Ed.),
595 Chichester, NewYork, Weinheim, Brisbane, Singapore, Toronto.

Figure legends

Figure 1: Flow diagram of the processing and chill chain of cooked, pasteurised and chilled courgettes purée (Guinebretière et al., 2003).

Figure 2: Mean proportion of each *B. cereus* genetic group relatively to the whole *B. cereus* population in 2000 batches of 300kg in (A) uncooked and (B) cooked courgettes (estimated by our exposure model).

^a number of the considered *B. cereus* genetic group: mean % of the group in the whole *B. cereus* population.

Figure 3: Mean concentration (log cfu/g) of the whole *B. cereus* population (Total) and per genetic group (II, III, IV, V, VI and VII) on the axes at various stages of the processing chain: initial contamination of raw courgettes (□), after cooking (△), after mixing and partitioning (▲), after pasteurisation (×) and at the end of home storage in refrigerators (■). Only units containing at least one spore of the genetic group were considered to calculate the mean concentration. Units were either the whole batch of 344kg before partitioning or the packages of 400g after partitioning.

Figure 4: Mean proportion of each genetic group relatively to the whole *B. cereus* population (A) after mixing and partitioning, (B) after pasteurisation and (C) at the end of home storage in refrigerators.

^a number of the considered *B. cereus* genetic group: mean % of the group in the whole *B. cereus* population.

^B (IV, V, VI) = (0.006%, 0.01%, 0.04%)

617 ^c (IV, V, VI) = (0.02%, 0.08%, 0.02%).

618 **Figure 5:** Joint concentrations (log cfu/g) of genetic group II (a psychrotrophic group)
619 and genetic group III (a mesophilic group) at the end of home storage in refrigerators.

620

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Table 1: Some characteristics of strains belonging to the genetic groups of *Bacillus cereus* defined by Guinebretière et al. (2008).

Genetic group	Growth at			Association to	
	7°C	10°C	43°C	cases of foodborne poisonings	currently defined species or group
I	No	Yes*	No*	No	<i>B. pseudomycoïdes</i>
II	Yes*	Yes	No	Yes	nd
III	No	No	Yes	Yes	Emetic <i>B. cereus</i> strains
IV	No	Yes	Yes*	Yes	nd
V	No	Yes	No	Yes	nd
VI	Yes	Yes	No	No	<i>B. weihenstephanensis</i> , <i>B. mycoïdes</i>
VII	No	No	Yes	Yes	nd

* for most of the strains.

nd: not defined

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Table 2: Variability distributions of time and temperature for the stages of the food pathway derived from industrial data, industrial expert's opinion or literature data.

Stage	Time t or t_{eq}	Temperature T (°C)	Reference
Cooking	^a E(1/161.4) min	$T = T_r = 90$	Company own data
Blending	^c U(15,20) min	U(80,90)	Company expert's opinion
Time interval	^d $\Gamma(1.85, 0.5)$ h	$\geq$ ^e N(45.6, 8.5) on [^f 24.3, 59.8]	Company own data
Pasteurisation	E(1/4) min	$T = T_r = 90$	Company own data
Chill chain	U(3,5) days and U(1,7) days	N(4,1) on [2;5]	Company expert's opinion
Refrigerated road transport	^g BP(18,36,72) h	BP(4,4.01,8)	Nauta, 2003
Chill chain at retail			
80%	U(0,14-BP(18,36,72)) days	N(4,2.17)	Nauta, 2003
20%	14-BP(18,36,72)+U(0,7) days	N(4,2.17)	Nauta, 2003
Home transportation	$\Gamma(5.24, 8.17)$ min	BP(4,10,25)	Nauta, 2003
Home storage in refrigerator	E(3/(^h SBD- ⁱ PD)) days	N(6.64, 2.35) [profile	Nauta, 2003
SBD	21 days	"Europe"]	Nauta, 2003

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^aE() is the Exponential distribution with one parameter (mean).

^bT_r is the reference temperature.

^cU() is the Uniform distribution with two parameters (inferior and superior limits).

^dΓ() is the Gamma distribution with two parameters (the first is the shape parameter and the second is the scale parameter).

^eN() is the normal distribution with two parameters (mean and standard deviation), eventually truncated on the interval [].

^fThe temperatures are recorded by the company at the end of the period.

^gBP() is the BetaPert distribution with 3 parameters (minimum, most likely and maximum values).

^hSBD is the Sell By Date.

ⁱPD is the Purchase Date.

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Table 3: Link between the stage in the food pathway and the microbial basic biological process of *B. cereus*.

Stage of the food pathway	Microbial basic biological process of <i>B. cereus</i>
Cooking	Inactivation of spores
Blending	Inactivation of spores
Time interval	Germination of spores
Pasteurisation	Total elimination of vegetative cells and inactivation of spores
Chill storage at the company	Germination of spores and growth of vegetative cells
Refrigerated road transport	Growth of vegetative cells
Chill storage at retail	Growth of vegetative cells
Home transportation	Growth of vegetative cells
Home storage in refrigerator	Growth of vegetative cells

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Table 4: Minimum (Min), most-likely (MI) and maximum (Max) values of Beta-Pert distributions for the initial spore contamination (log cfu/g) for each *B. cereus* genetic group (adapted from Guinebretière et al., 2003) in unprocessed courgettes, milk proteins and starch (in bold, groups detected by enrichment in Guinebretière et al., 2003).

Genetic group	Unprocessed courgettes			Milk proteins			Starch		
	Min	MI	Max	Min	MI	Max	Min	MI	Max
II	-2	-0.8	0.4	-5.6	-3.9	-2.1	-4.2	-2.8	-1.3
III	-4.4	-3.2	-2	-2.1	-0.4	1.4	-4.2	-2.8	-1.3
IV	-4.4	-3.2	-2	-2.1	-0.4	1.4	-1.3	0.2	1.6
V	-4.4	-3.2	-2	-5.6	-3.9	-2.1	-4.2	-2.8	-1.3
VI	-2	-0.8	0.4	-5.6	-3.9	-2.1	-4.2	-2.8	-1.3
VII	-4.4	-3.2	-2	-5.6	-3.9	-2.1	-4.2	-2.8	-1.3

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Table 5: Variability distribution per batch of thermal inactivation parameters δ and p_s for the *B. cereus* genetic groups (II, III, IV, V, VI and VII) and uncertainty distribution per batch of thermal inactivation parameter z for the whole *B. cereus* population (ALL).

Genetic group	z (°C)	δ_s (min)			p_s		
		Minimum	Most likely ^a	Maximum	Minimum	Most likely ^a	Maximum
II	Data not available	2.3	30.5	76.3	0.5	1	1.4
III	Data not available	2.4	39	171.7	0.01	0.6	7
IV	Data not available	3.2	25.7	148.3	0.2	0.7	2.7
V	Data not available	14.8	20.5	26.2	0.6	0.8	1
VI	Data not available	0.01	1.7	10	0.2	0.4	0.8
VII	Data not available	40.8	90.3	139.9	1.2	1.3	1.4
ALL	^b logN(2.5, 1.7) on [6.5;14.5]						

^aThe most likely value is the median of observed values.

^blogN(m,s) on [a;b] is the log-normal distribution with two parameters (m: mean and s: standard deviation) truncated on the interval [a; b].

Table 6: Mean cardinal temperatures ($T_{\min}^s, T_{opt}^s, T_{\max}^s$) estimated from the range of growth temperatures recorded for each genetic *B. cereus* group from II to VII (Guinebrerière et al., 2008) and uncertainty distribution of $\log_{10} N_{\max}$ and μ_{opt} per batch for the whole *B. cereus* population (ALL).

Genetic group	$\log_{10} N_{\max}$ (log cfu/g)	μ_{opt} (h ⁻¹)	$T_{\min}^s$ (°C)	T_{opt}^s (°C)	$T_{\max}^s$ (°C)	Source
II	Data not available	Data not available	1.8	34.9	39.4	Expert's opinion
III	Data not available	Data not available	6.0	39.8	46.9	Sym'Previus (http://www.symprevius.net/), Auger et al., 2008
IV	Data not available	Data not available	4.4	39.9	46.5	Sym'Previus, Auger et al., 2008
V	Data not available	Data not available	3.0	35.4	39.4	Sym'Previus
VI	Data not available	Data not available	0.5	34.3	38.2	Sym'Previus, Auger et al., 2008
VII	Data not available	Data not available	12.1	41.4	56.5	Auger et al., 2008
ALL	^a N(8.5, 0.6)	^b BP(0.58,0.76,1.04)				Choma et al., 2000

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^aN(m, s) is the normal distribution with 2 parameters (m: mean and s: standard deviation).

^bBP() is the BetaPert distribution with 3 parameters (minimum, most likely and maximum values).

Table 7: Prevalence of units ^a containing at least one *B. cereus* spore (%) from each genetic group (II, III, IV, V, VI, or VII) at main key stages of the food pathway.

<i>B. cereus</i> genetic group	<i>Prevalence (%) after the designated processing stage</i>				
	Initial contamination of 300-kg batches of raw courgettes	After cooking of 300-kg batches of raw courgettes	After mixing with ingredients and partitioning into 400-g packages	After pasteurisation of 400-g packages	At the end of home storage in refrigerators
II	100%	62%	33%	19%	19%
III	100%	54%	81%	37%	37%
IV	100%	49%	100%	82%	82%
V	100%	30%	4%	0.1%	0.1%
VI	100%	47%	6%	0.1%	0.1%
VII	100%	65%	9%	0.6%	0.6%
Total	100%	83%	100%	87%	87%

^a The prevalences of units are computed on 2000 batches or 200000 packages.

Table 8: Confidence interval (CI) at 95% of the mean *B. cereus* concentration (log cfu/g) per genetic group and per unit^a at main key stages of the food pathway.

B. <i>cereus</i> genetic group	CI at 95 % of mean <i>B. cereus</i> concentration (log cfu/g) after the designated processing stage				
	Initial contamination of 300-kg batches of raw courgettes	After cooking of 300-kg batches of raw courgettes	After mixing with ingredients and partitioning into 400-g packages	After pasteurisation of 400-g packages	At the end of home storage in refrigerators
II	[-1.5;0]	[-5.5;-0.6]	[-2.6;-0.6]	[-2.6;-0.8]	[-2.6;6.7]
III	[-4;-2.4]	[-5.5;-2.8]	[-2.6;-1]	[-2.6;-1.1]	[-2.6;0]
IV	[-4;-2.4]	[-5.5;-2.9]	[-2.1;-0.4]	[-2.6;-0.6]	[-2.6;1.7]
V	[-4;-2.5]	[-5.5;-3.1]	[-2.6;-2.3]	[-2.6;-2.6]	[-2.6;3.4]
VI	[-1.6;0]	[-5.5;-1.5]	[-2.6;-1.8]	[-2.6;-2.3]	[-2;7.8]
VII	[-4;-2.4]	[-5.5;-2.7]	[-2.6;-2.1]	[-2.6;-2.1]	[-2.6;-2.1]
Total	[-1;0.2]	[-5.5;-0.6]	[-1.8;-0.4]	[-2.6;-0.5]	[-2.6;3.5]

^aOnly units containing at least one spore of the genetic group were considered to calculate the mean concentration.