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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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## 13 Abstract

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

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

## 35 Keywords

36 *Bacillus cereus*, Bacterial diversity, Quantitative microbial risk assessment,  
37 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*

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

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

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

## 88 2. Materials and methods

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

### 99 2.1 Data on the initial contamination in the vegetable and its ingredients

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

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

## 116 2.2 The food pathway

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

123 During processing, fresh courgettes were firstly trimmed and peeled, washed,  
124 then steam cooked for a mean equivalent time at 90°C of 161.4 min and blended for  
125 15 min at a temperature of 80 °C (Table 2). The mixing process included 300 kg of  
126 cooked and blended courgettes and 44 kg ingredients (29 kg of UHT dairy cream and  
127 salt, 10 kg of starch, and 5 kg of milk proteins) and produced a courgette purée batch.  
128 Starch and milk proteins were a second source of bacterial contamination  
129 (Guinebretière et al., 2003).

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

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

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

### 147 2.3 Microbial basic processes

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

#### 153 2.3.1 Thermal inactivation model

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

$$156 \log_{10} \left( \frac{N_t^S}{N_0^S} \right) = - \left( \frac{t}{\delta'_S} \right)^{p_s}$$

157 (1)

158 where

159 -  $t$  (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 -  $\delta'_s$  (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):

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

(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 -  $\delta_s^r$  (min) is the calculated  $\delta$ -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  $\delta$ .

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

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

### 188 2.3.2 Spore germination

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

197 Spores might germinate during the time interval between partitioning and  
198 pasteurisation, as the recorded temperature decreased enough. Germinated spores  
199 were then assumed to be killed during pasteurisation, as the temperature during this  
200 process was substantially higher than 70 °C for 15 min. In other words, populations of  
201 the genetic groups with the highest germination rate will be the most reduced by  
202 pasteurisation. Within the range of the considered temperatures, the germination was  
203 assumed to occur below 45°C for spores of groups III, IV, V and VII, below 42°C for  
204 spores of group II, and only below 37°C for spores of group VI. When germination  
205 occurs, it was also assumed to be instantaneous, and that 90 % of the spores of each  
206 group germinate (Johnson et al., 1983; Nguyen-the and Carlin, unpublished data).  
207 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 -  $\mu_{\max}^S$  ( $\text{h}^{-1}$ ) is the maximum growth rate for genetic group  $s$ ,
- 225 -  $\lambda$  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 -  $\mu_{opt}$  ( $\text{day}^{-1}$ ) 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:

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

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

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

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

289 Table 5 shows the heat-resistance parameters of the *B. cereus* genetic groups  
290 estimated from a collection of strains representative of groups II ( $n = 7$ ), III ( $n = 35$ ),  
291 IV ( $n = 27$ ), V ( $n = 2$ ), VI ( $n = 11$ ) and VII ( $n = 2$ ). Parameter estimation and strain  
292 typing were performed in previous works (Carlin et al., 2006; Guinebretière et al.,  
293 2008). In our exposure model, a Beta-Pert distribution was used for  $p_s$  (see Eq. 1) and

294  $\delta_s^r$  (see Eq. 2). For some heat resistant strains (group III), curve fitting did not allow a  
295 proper determination of  $\delta$ -values. For those strains, a  $\delta$ -value of 180 min was chosen  
296 by default. The parameters of the distribution of  $z$  values (see Eq. 2 and Table 5),  
297 assumed to be independent of the genetic groups, were estimated from 94 literature  
298 data (Gilbert et al., 1974; Bradshaw et al., 1975; Johnson et al., 1983; Rajkowski &  
299 Mikolajcik, 1987; Picoche et al., 1993; Mazas et al., 1995; ICSMF, 1996; Faille et al.,  
300 1997; Gonzalez et al., 1997; Gaillard et al., 1998 (a), 1998 (b); Fernandez et al., 1999;  
301 Gonzalez et al 1999; Mazas et al., 1999; Mafart et al., 2002) and followed a log-  
302 normal distribution truncated on a temperature interval. The median was 8.3 °C. For  
303 cooking and pasteurisation, an exponential distribution was fitted to the equivalent  
304 times  $t_{eq}$  computed from Equation (3) on 5 and 40 time-temperature profiles recorded  
305 by the company, respectively. In this computation, an uncertainty on  $z$  value (see Eq.  
306 2 and Table 5) was inserted.

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

318 assumed to be a Beta-Pert distribution, with parameters estimated to  $0.58 \text{ h}^{-1}$ ,  $0.70 \text{ h}^{-1}$ ,  
319  $1.04 \text{ h}^{-1}$ , respectively (Table 6). For  $\log N_{\max}$ , Choma et al. (2000) studied a broad  
320 range of products and reported maximal numbers of *B. cereus* at the end of  
321 refrigeration storage reaching  $8.5 \pm 0.6 \text{ log CFU/g}$ . Thus, we assumed that this last  
322 parameter followed a normal distribution with a mean of  $8.5 \text{ log CFU/g}$  and a  
323 standard deviation of  $0.6 \text{ log CFU/g}$  (Table 6).

324 Only packs of purée units containing *B. cereus* represent a hazard for  
325 consumers. The relevant information to assess consumer exposure is therefore the  
326 concentration of *B. cereus* in positive units, combined with the prevalence of positive  
327 units, and addressing a null risk to negative units. At main key stages of the food  
328 pathway (initial contamination of 300 kg batches of raw courgettes, after cooking of  
329 300 kg batches, after mixing with ingredients and partitioning into 400 g packages,  
330 after pasteurisation of 400 g packages and at the end of home storage in  
331 refrigerators), the prevalences of packages containing at least one *B. cereus* spore,  
332 whatever the genetic group, were computed, and the bacterial load ( $\text{log CFU/g}$ ) of the  
333 contaminated units (batch or package) per genetic group was calculated, taking into  
334 account units containing at least one CFU of this group. The proportions of each *B.*  
335 *cereus* genetic group to the whole *B. cereus* population in the batches or packages  
336 were also calculated, considering all units.

### 337 3. Results

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



343 (batch or packages) and also the corresponding 95% confidence intervals (CI) per  
344 genetic group, reflecting the uncertainty assumed in the model. There was a ranking  
345 of the genetic groups as a function of their resistance to cooking (Fig. 3). The  
346 mesophilic group VII was the most resistant of all groups, and among the  
347 psychrotrophic groups, group II was the most resistant, whereas group VI was the  
348 most sensitive to heat. This resulted in a major decrease in the number of the  
349 psychrotrophic group VI after cooking in positive units. However, their prevalence  
350 (47%) in units after cooking was similar to that of the more heat resistance group IV  
351 (49%), because group VI was more abundant in the uncooked courgette (Table 7).  
352 The upper bounds of CI were relatively unchanged by cooking (Table 8). The lower  
353 bound of CI corresponded to one spore in a 300 kg batch. Among the psychrotrophic  
354 groups, group II prevailed, because of heat-resistance higher than that of group VI  
355 (Fig. 2B). The proportions of groups III, IV, and VII, rare in uncooked vegetables  
356 (Fig. 2A), increased after cooking (Fig. 2B) likely because of their high resistance to  
357 heat and the corresponding decrease from 48.3% to 13.1% of the proportion of the  
358 more heat sensitive group VI.

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

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

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

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

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

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

#### 411 4. Discussion

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

418 group relatively to the whole *B. cereus* population was strongly influenced by  
419 processing, storage and contamination routes. By integrating the various components  
420 of a bacterial population in a single model, instead of running various models  
421 independently for each component, we were able to simulate the prevalence of the  
422 genetic groups and the composition of the bacterial population (proportion between  
423 the different groups in the whole *B. cereus* population) at any stage of the food  
424 pathway. The mean proportion of all genetic groups cannot be interpreted  
425 independently of bacterial concentration in contaminated packages and of prevalence  
426 of each genetic group. According to the exposure model, the most psychrotrophic  
427 group VI was eliminated from most of the packages because of low heat resistance,  
428 but it reached the highest predicted *B. cereus* populations in some packages (indicated  
429 by the upper bounds of confidence intervals). In contrast, the prevailing groups were  
430 the mesophilic ones, which better resisted the heat treatments, but with a much lower  
431 growth potential during refrigeration storage. Their concentrations rarely exceeded  
432  $10^3$  CFU/g, which usually is not a concern for food safety (Granum, 2007). A proper  
433 risk assessment should also include the virulence properties of each genetic group.  
434 Most of the strains related to cases or outbreaks of *B. cereus* foodborne poisoning  
435 belong to groups III and IV (Guinebretière et al. 2008). Considering psychrotrophic  
436 groups, Stenfors et al. (2002) observed that *B. weihenstephanensis*, which belongs to  
437 *B. cereus* group VI (Guinebretière et al., 2008), was poorly cytotoxic on Vero cells  
438 compared to other *B. cereus*. Similarly, Choma et al. (2000) found the most  
439 psychrotrophic strains of *B. cereus* the least toxic on Caco2 cells. According to  
440 Svensson et al. (2007), most of the strains isolated from refrigerated pasteurized milk  
441 were *B. weihenstephanensis* (i.e. *B. cereus* group VI) and produced very low amount  
442 of enterotoxins. These works strongly suggest that *B. cereus* group VI is less prone to

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

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596 **Figure legends**

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

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

602 <sup>a</sup> number of the considered *B. cereus* genetic group: mean % of the group in the whole  
603 *B. cereus* population.

604 **Figure 3:** Mean concentration (log cfu/g) of the whole *B. cereus* population (Total)  
605 and per genetic group (II, III, IV, V, VI and VII) on the axes at various stages of the  
606 processing chain: initial contamination of raw courgettes (□), after cooking (△), after  
607 mixing and partitioning (▲), after pasteurisation (×) and at the end of home storage  
608 in refrigerators (■). Only units containing at least one spore of the genetic group were  
609 considered to calculate the mean concentration. Units were either the whole batch of  
610 344kg before partitioning or the packages of 400g after partitioning.

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

614 <sup>a</sup> number of the considered *B. cereus* genetic group: mean % of the group in the whole  
615 *B. cereus* population.

616 <sup>B</sup> (IV, V, VI) = (0.006%, 0.01%, 0.04%)

617 <sup>c</sup> (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.

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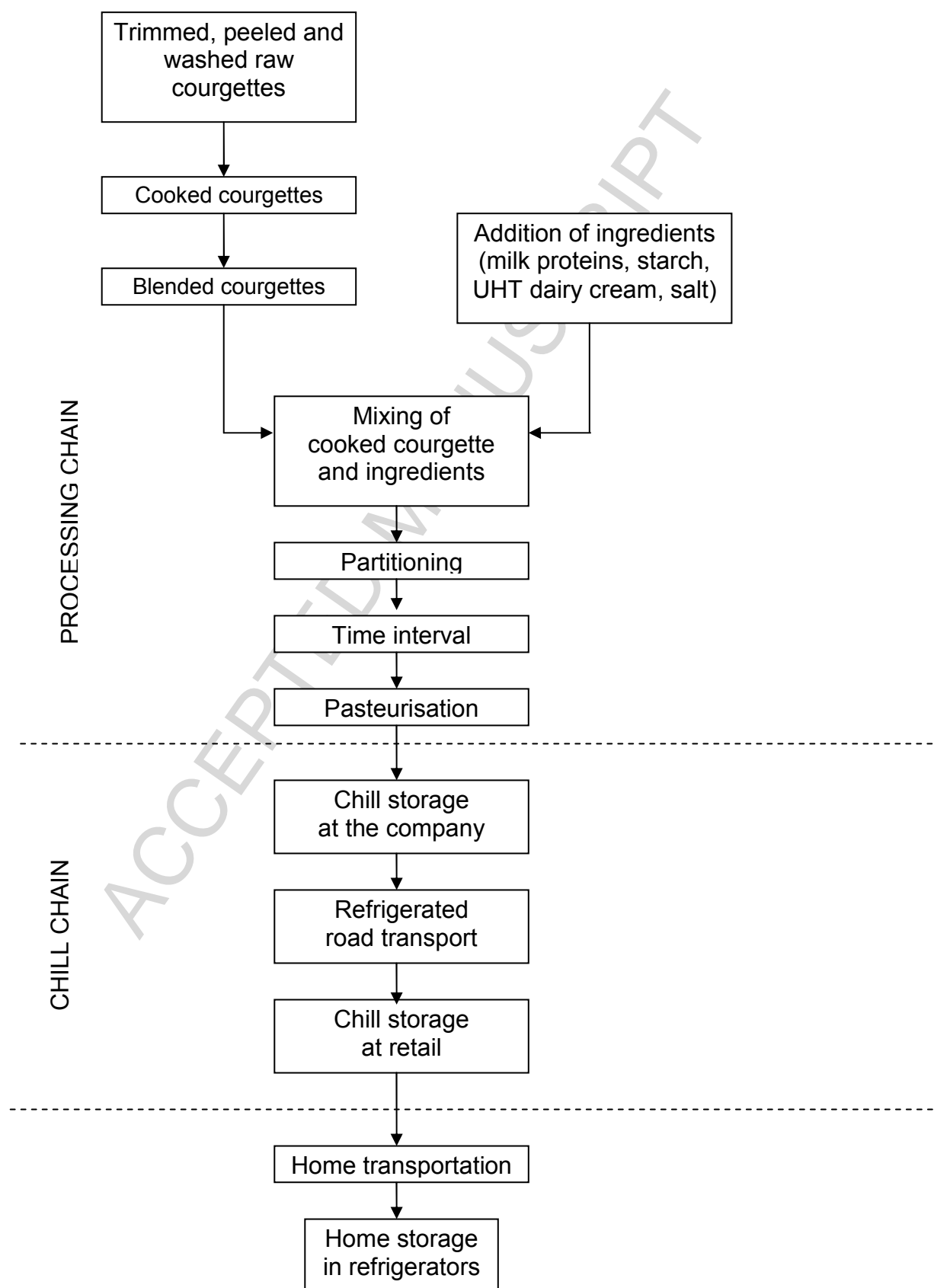


Figure 1

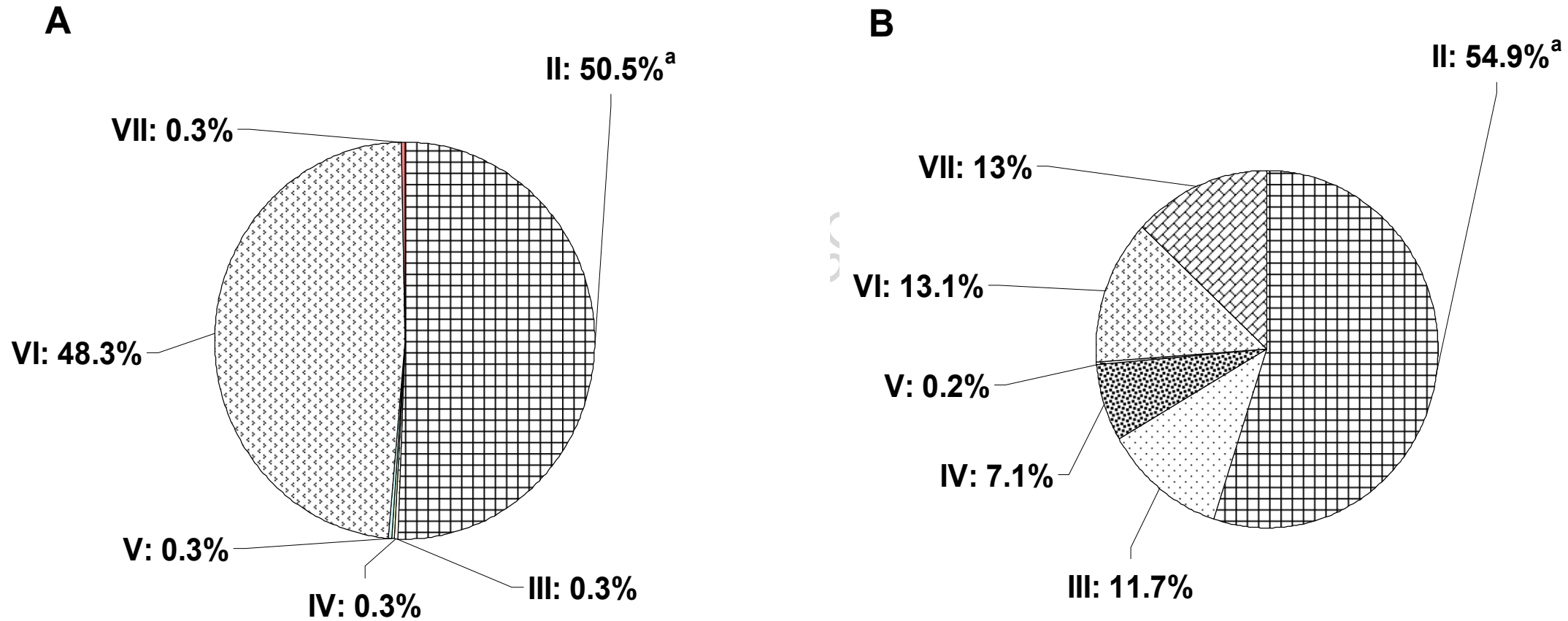


Figure 2

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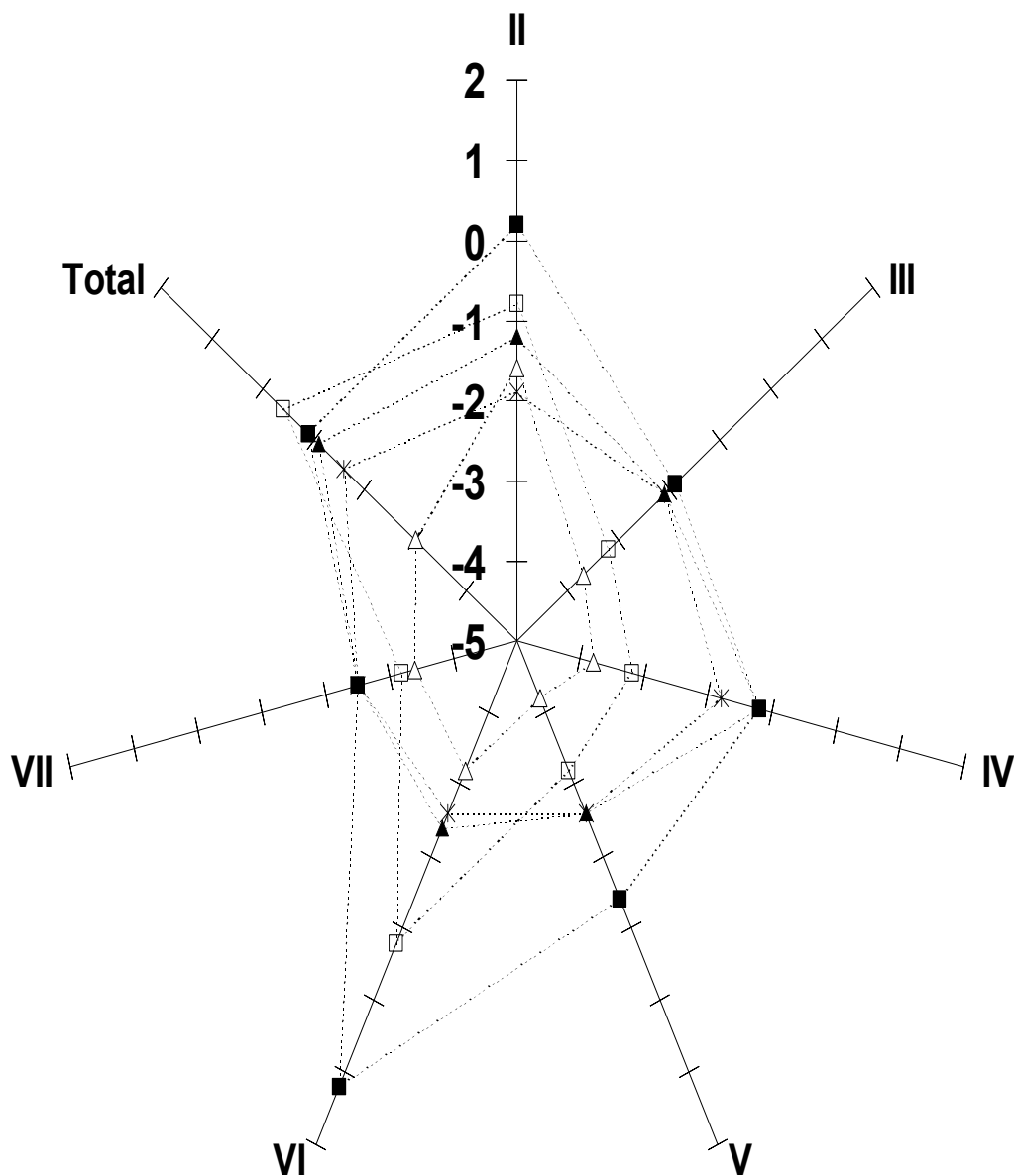


Figure 3

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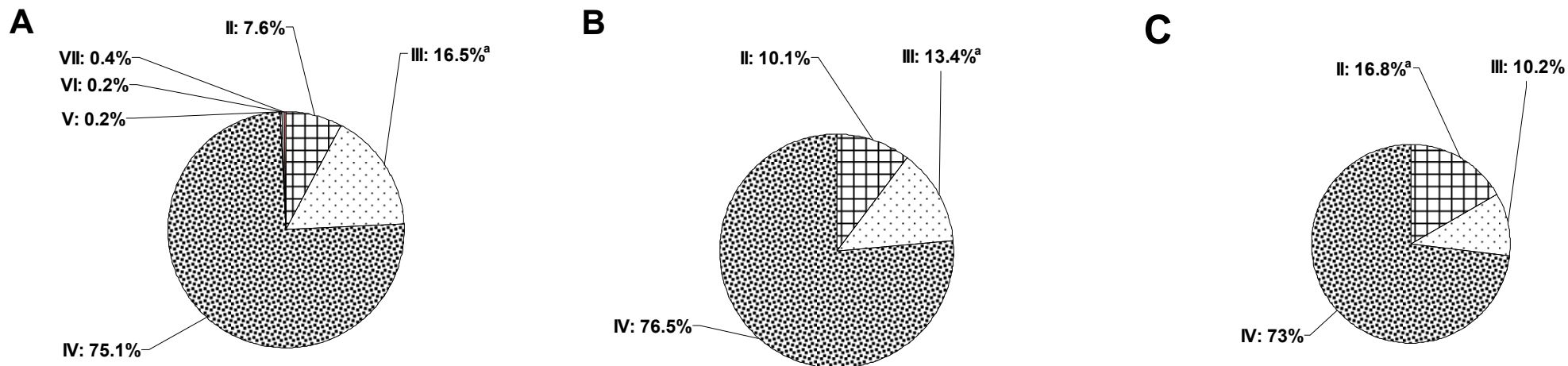
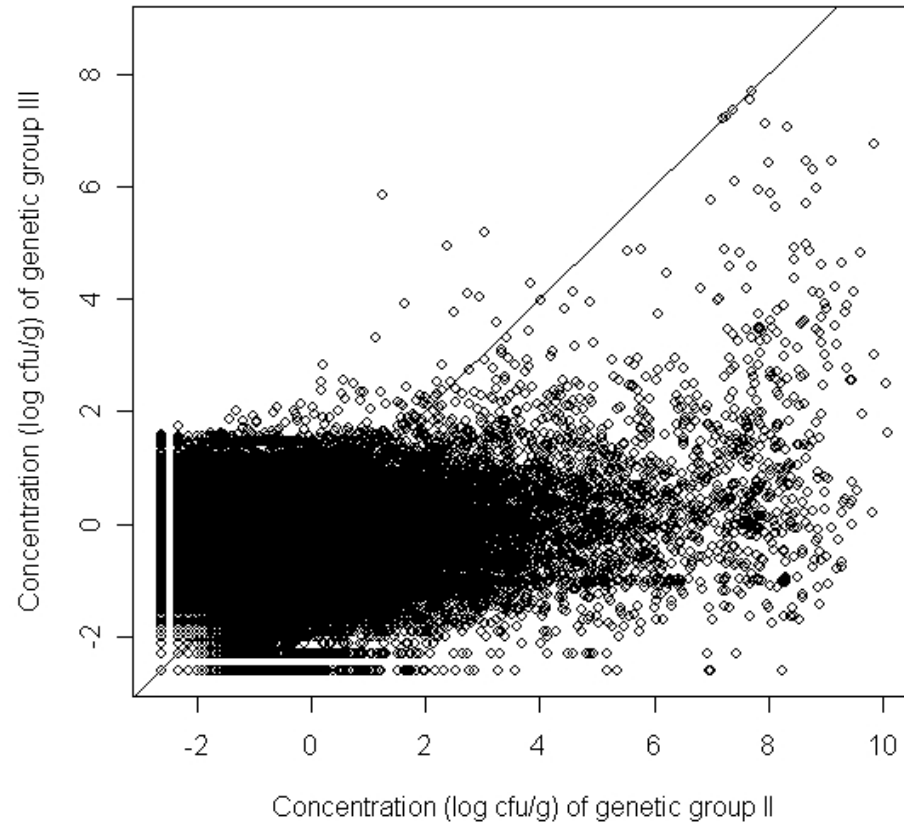


Figure 4

*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

**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	<sup>a</sup> E(1/161.4) min	T= <sup>b</sup> T <sub>r</sub> =90	Company own data
Blending	<sup>c</sup> U(15,20) min	U(80,90)	Company expert's opinion
Time interval	<sup>d</sup> Γ(1.85,0.5) h	≥ <sup>e</sup> N(45.6,8.5) on [ <sup>f</sup> 24.3,59.8]	Company own data
Pasteurisation	E(1/4) min	T=T <sub>r</sub> =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	<sup>g</sup> 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	Γ(5.24,8.17) min	BP(4,10,25)	Nauta, 2003
Home storage in refrigerator	E(3/( <sup>h</sup> SBD- <sup>i</sup> PD)) days	N(6.64,2.35) [profile	Nauta, 2003
SBD	21 days	"Europe"]	Nauta, 2003

Comment citer ce document :

Afchain, A. L., Carlin, F., Nguyen, C., Albert, I. (2008). Improving quantitative exposure assessment by considering genetic diversity of *B. cereus* in cooked, pasteurised and chilled foods. International Journal of Food Microbiology, 128 (1), 165-173. DOI : 10.1016/j.ijfoodmicro.2008.07.028

<sup>a</sup>E( ) is the Exponential distribution with one parameter (mean).

<sup>b</sup>T<sub>r</sub> is the reference temperature.

<sup>c</sup>U( ) is the Uniform distribution with two parameters (inferior and superior limits).

<sup>d</sup>Γ( ) is the Gamma distribution with two parameters (the first is the shape parameter and the second is the scale parameter).

<sup>e</sup>N( ) is the normal distribution with two parameters (mean and standard deviation), eventually truncated on the interval [ ].

<sup>f</sup>The temperatures are recorded by the company at the end of the period.

<sup>g</sup>BP( ) is the BetaPert distribution with 3 parameters (minimum, most likely and maximum values).

<sup>h</sup>SBD is the Sell By Date.

<sup>i</sup>PD is the Purchase Date.

**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	<b>-2</b>	<b>-0.8</b>	<b>0.4</b>	-5.6	-3.9	-2.1	-4.2	-2.8	-1.3
III	-4.4	-3.2	-2	<b>-2.1</b>	<b>-0.4</b>	<b>1.4</b>	-4.2	-2.8	-1.3
IV	-4.4	-3.2	-2	<b>-2.1</b>	<b>-0.4</b>	<b>1.4</b>	<b>-1.3</b>	<b>0.2</b>	<b>1.6</b>
V	-4.4	-3.2	-2	-5.6	-3.9	-2.1	-4.2	-2.8	-1.3
VI	<b>-2</b>	<b>-0.8</b>	<b>0.4</b>	-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  $\delta_s$  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)	$\delta_s$ (min)			$p_s$		
		Minimum	Most likely <sup>a</sup>	Maximum	Minimum	Most likely <sup>a</sup>	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	<sup>b</sup> logN(2.5, 1.7) on [6.5;14.5]						

<sup>a</sup>The most likely value is the median of observed values.

<sup>b</sup>logN(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 (Guinebretière et al., 2008) and uncertainty distribution of  $\log_{10} N_{\max}$  and  $\mu_{opt}$  per batch for the whole *B. cereus* population (ALL).**

Genetic group	$\log_{10} N_{\max}$ (log cfu/g)	$\mu_{opt}$ ( $h^{-1}$ )	$T_{\min}^s$ ( $^{\circ}C$ )	$T_{opt}^s$ ( $^{\circ}C$ )	$T_{\max}^s$ ( $^{\circ}C$ )	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 ( <a href="http://www.symprevius.net/">http://www.symprevius.net/</a> ), 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	<sup>a</sup> N(8.5, 0.6)	<sup>b</sup> BP(0.58,0.76,1.04)				Choma et al., 2000

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<sup>a</sup> $N(m, s)$  is the normal distribution with 2 parameters ( $m$ : mean and  $s$ : standard deviation).

<sup>b</sup> $BP()$  is the BetaPert distribution with 3 parameters (minimum, most likely and maximum values).

**Table 7: Prevalence of units <sup>a</sup> 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%

<sup>a</sup> 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<sup>a</sup> at main key stages of the food pathway.**

B. cereus 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]

<sup>a</sup>Only units containing at least one spore of the genetic group were considered to calculate the mean concentration.