

## Quantitative assessment of the risk of microbial spoilage in foods. Prediction of non-stability at 55 °C caused by *Geobacillus* *stearothermophilus* in canned green beans

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### A B S T R A C T

Microbial spoilage of canned foods by thermophilic and highly heat-resistant spore-forming bacteria, such as *Geobacillus stearothermophilus*, is a persistent problem in the food industry. An incubation test at 55 °C for 7 days, then validation of biological stability, is used as an indicator of compliance with good manufacturing practices. We propose a microbial risk assessment model predicting the percentage of non-stability due to *G. stearothermophilus* in canned green beans manufactured by a French company. The model accounts for initial microbial contaminations of fresh unprocessed green beans with *G. stearothermophilus*, cross-contaminations in the processing chain, inactivation processes and probability of survival and growth. The sterilization process is modeled by an equivalent heating time depending on sterilization value  $F_0$  and on *G. stearothermophilus* resistance parameter  $z_T$ . Following the recommendations of international organizations, second order Monte-Carlo simulations are used, separately propagating uncertainty and variability on parameters. As a result of the model, the mean predicted non-stability rate is of 0.5%, with a 95% uncertainty interval of [0.1%; 1.2%], which is highly similar to data communicated by the French industry. A sensitivity analysis based on Sobol indices and some scenario tests underline the importance of cross-contamination at the blanching step, in addition to inactivation due to the sterilization process.

#### Keywords:

*Geobacillus stearothermophilus*

Prevalence

Cross contaminations

Process risk model

Second order Monte Carlo simulation

Sensitivity analysis

### 1. Introduction

Canned foods have an excellent safety record due to the inactivation of pathogenic bacteria. The highest risk for public health, due to *Clostridium botulinum*, is controlled in low acid foods (pH > 4.5) by the application of the 'botulinum cook', which is defined as equivalent to 3 min heating at 121 °C. However the spoilage of canned foods because of the persistence of non-pathogenic thermophilic and highly heat-resistant spore-forming bacteria is still an industrial and economical risk (Logan and De Vos, 2009; Burgess et al., 2010; Prevost et al., 2010). The stability after incubation at 55 °C for 7 days (French standard NF V08-408 (AFNOR, 1997)) of canned foods is used as a hygiene indicator and as a norm for exportation to hot-climate regions as required by some national regulations, such as that of France (Anonymous, 1997). Among thermophilic bacteria, *Geobacillus stearothermophilus* is recognized as a major source of spoilage in canned foods and is frequently detected in cans presenting defects after a 7-day incubation at 55 °C (André et al., 2013).

The canning process combines several operations allowing contamination, inactivation and/or growth of micro-organisms. The final stability is the result of the individual contributions of the processing operations. In this work, the fate of *G. stearothermophilus* is modeled along a canned green bean processing chain to predict the rate of non-stability due to *G. stearothermophilus* after the standard incubation test at 55 °C. A quantitative microbial risk assessment was used, with a probabilistic approach accounting for sources of variability and uncertainty. Variability represents the natural heterogeneity of a factor, coming for instance from heterogeneity between bacterial strains, and is irreducible by nature. Uncertainty comes from a reducible lack of knowledge on the true value of a parameter and for instance from a lack of information, sampling or measurement errors (Pouillot and Delignette-Muller, 2010; Vose, 2000). Risk variability distributions and evaluation of uncertainty associated with this spoilage risk were obtained using a two-dimensional Monte Carlo simulation separately propagating uncertainty and variability through the model (Vose, 2000; Mokhtari and Frey, 2005; Pouillot et al., 2007; Pouillot and Delignette-Muller, 2010). This distinction is useful for risk managers and has been recommended by international organizations since a few years (Codex Alimentarius Commission, 1999; European Commission, 2003).

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The aims of this study were to predict the risk of spoilage in canned foods and to evaluate the reliability of the prediction. Next, the impact on the final predictions of several risk management options or of the potential consequences of the uncertainty of some assumptions was tested using what-if scenarios. A sensitivity analysis using Sobol indices allowed the detection of the most influential risk parameters.

## 2. Material and methods

### 2.1. Overview of the model

A modular process risk model (Nauta, 2001) has been developed. This was made up of several steps following the contamination of green beans with *G. stearothermophilus* from the fresh unprocessed product to the end of the industrial canning process. *G. stearothermophilus* growth leading to the spoilage of canned green beans is considered during a 7-day incubation test at 55 °C. The different steps of the food chain and the basic microbial process impacting the fate of *G. stearothermophilus* at each step were described in Table 1. Basically, the risk model accounts for (i) the inactivation of *G. stearothermophilus* during heating processes (blanching and sterilization), depending on processing time and temperature and on pH of canned food, (ii) cross-contamination at blanching and brining and (iii) the probability of survival and growth to cause spoilage after incubation. Expert opinion, literature data, information on the food process and specifically collected data, such as prevalence of *G. stearothermophilus* in green bean samples, have been used to build the model.

Processing of green beans for canning is almost continuous, with no clear identification of batches. Consequently (and also for model simplification) the modeled unit is one can containing 445 g of green beans filled with 405 g of covering brine, leading to a final weight of 850 g. Concentrations (or continuous numbers of CFU) of *G. stearothermophilus* were used at all steps of the model, except at the germination step which was modeled using discrete numbers (step occurring just after sterilization, with contaminations at low level). The outputs of the model are (i) the concentrations of *G. stearothermophilus* after sterilization and after incubation tests and (ii) the non-stability rate, i.e. the percentage of green bean cans presenting a defect at the end of the incubation test.

### 2.2. Determination of *G. stearothermophilus* concentrations at different processing steps

Concentrations of Aerobic Thermophilic Spores (ATS) were determined in samples of green beans collected on the chains of two processing plants during the 2-month production periods of years 2011 and 2012. Samples of 10 g were collected from raw green beans before blanching (number of samples  $n = 95$ ), and from blanched green beans immediately before can filling ( $n = 93$ ). Samples of 100 ml

were collected from blanching water ( $n = 45$ ) and from covering brine ( $n = 99$ ). All samples were stored frozen at  $-18$  °C for 1 to 4 weeks, thawed at room temperature, homogenized, then treated at 100 °C for 10 min to eliminate vegetative cells (French Standard NFV08-602) (AFNOR, 2011). Serial decimal dilutions were made in tryptone salt buffer and 1 ml was mixed with 25 ml melted BCP agar and incubated at 55 °C for 2 days. When no colonies were detected, ATS counts were considered as left-censored data (i.e., under the threshold of detection: 10 CFU/g for solid samples or 1 CFU/ml for liquid samples). When colonies were detected, up to three colonies were randomly picked for identification of *G. stearothermophilus* using the molecular tool SporesTraQ™ (Prevost et al., 2010), in order to estimate the proportion (or ratio) of *G. stearothermophilus* among ATS.

### 2.3. Estimation of the green bean pH at different processing steps

The green bean pH at the blanching step was based on specific measurements on the process chain before sterilization. The pH of the sterilized product, in which growth will occur, was assumed identical to the pH of the covering brine after sterilization, which was measured by the canning processor according to the French standard NFV08-408 (AFNOR, 1997).

To estimate the pH during sterilization, cans (1/4 US size) were filled with 83 g of frozen blanched green beans and with 110 ml of covering brine made up of tap water and sodium chloride at 1% (wt./vol.). Cans were placed in an autoclave and heated at 120 °C. Cans were sampled at regular time intervals between 0 min and 15 min after the come-up-time. The pH of the brine and of blended green beans was measured using a pH meter calibrated at pH 4.0 and 7.0 buffer solutions (Sartorius, Aubagne, France). This procedure was applied on five samples of green beans for each time of treatment. A rapid equilibrium was shown between brine and green beans, and no difference was observed immediately after the come-up-time. Consequently the pH at equilibrium was considered as the pH to which *G. stearothermophilus* is subjected during sterilization.

### 2.4. Two-dimensional Monte Carlo simulation

Within the two-dimensional Monte Carlo simulation framework, uncertainty and variability were separately propagated through the model. According to expert opinions and data and modeling choices, parameters of the model were classified into four categories: fixed, variable, uncertain and both variable and uncertain, as previously detailed (Pouillot and Delignette-Muller, 2010). The simulations were made by first sampling in the uncertainty dimension (of sample size  $N_u$ ), then by sampling in the variability dimension (of sample size  $N_v$ ) conditionally to the sampled uncertain parameters (Vose, 2000; Pouillot et al., 2007; Pouillot and Delignette-Muller, 2010). This procedure leads to a global sample of size  $N_u \times N_v$ . The simulations were performed with  $N_u = 10,000$  and  $N_v = 100,000$ , using the R software (R Development Core Team, 2010).

### 2.5. Model and parameters

The overall model is represented by a directed acyclic graph (DAG) (Fig. 1), which gives the conditional dependencies between the model parameters.

#### 2.5.1. Initial contamination and cross-contamination sources

Log-normal (base e) variability distributions were adjusted on the measured ATS concentrations based upon maximum likelihood estimation, and parameter uncertainty was evaluated by non-parametric bootstrap using the R package fitdistrplus (Pouillot and Delignette-Muller, 2010). *G. stearothermophilus* ratio among ATS at blanching was significantly different from the ratio in blanching water and in covering

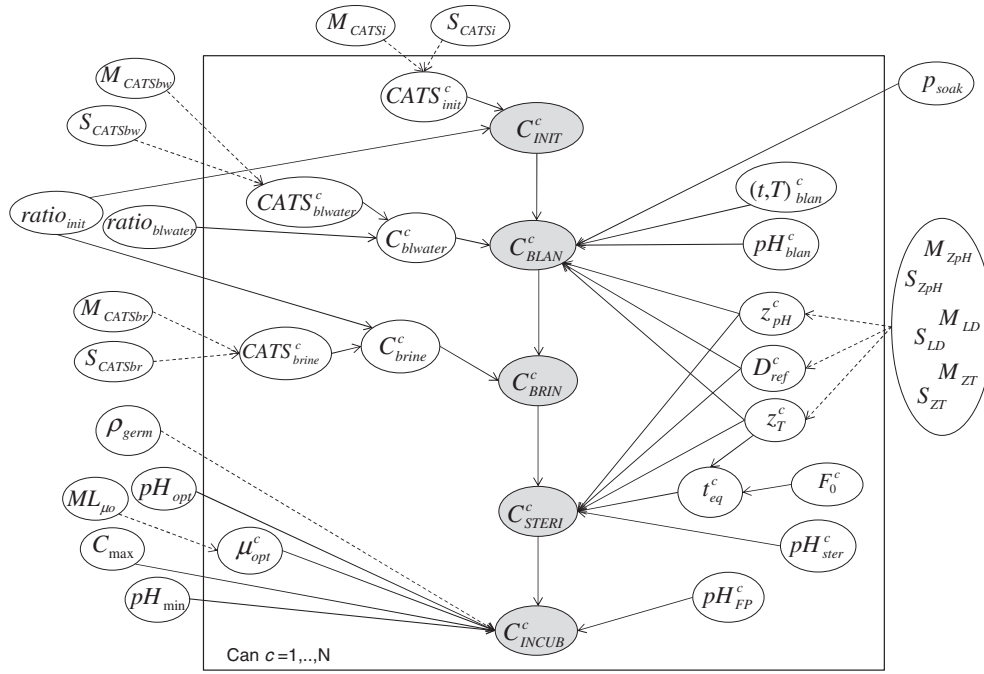
**Table 1**

Description of the food pathway (including the incubation test) and link with the modeled basic microbial processes impacting the fate of *G. stearothermophilus*.

Step X of the food pathway and of incubation test (abbreviation)	Microbiological process affecting <i>G. stearothermophilus</i>
Green bean harvesting (INIT)	Initial contamination
Blanching (BLAN)	Inactivation of spores, and cross contamination by blanching water
Canning and brining (BRIN)	Cross-contamination by recovery brine
Sterilization (STERI)	Inactivation of spores
Cooling (COOL)	None
Incubation test (INCUB)	Germination then growth

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**Fig. 1.** The global risk model represented by a directed acyclic graph (DAG). Model parameters are represented by nodes. The nodes are described in Table 2 and/or in Section 2.5. The principal nodes  $C_x$  are colored in gray; they denote the *G. stearothermophilus* concentrations at steps X of the food chain (see Table 1). Solid edges indicate deterministic links and dashed edges indicate stochastic links between nodes. Index c denotes the can numbers and specifies the can-dependent-variables. The correlation between the hyperparameters  $M_{ZT}$ ,  $M_{ZpH}$ ,  $M_{Dref}$ ,  $S_{ZT}$ ,  $S_{ZpH}$  and  $S_{Dref}$  is indicated by grouping in a single node.

brine, and this was considered in the model parameters. The corresponding adjusted distributions are presented in Table 2. The concentrations in green beans before filling were used to validate the model. As no significant correlation between ATS concentrations and *G. stearothermophilus* ratios was detected, the *G. stearothermophilus* concentration is given by:

$$C_x = CATS_x \cdot ratio_x \quad (1)$$

where  $C_x$  (resp.  $CATS_x$ ) (CFU/g) is the *G. stearothermophilus* (resp. ATS) concentration and  $ratio_x$  is the proportion of *G. stearothermophilus* among ATS in the contamination source  $x$ .  $x$  represents either the fresh green beans ( $x = init$ ) or the blanching water ( $x = blwater$ ) or brine ( $x = brine$ ).

### 2.5.2. Inactivation models

Spore inactivation was assimilated to first order kinetics and survival curves were classically considered as log-linear. The primary inactivation model is therefore described by:

$$\log_{10}(C(t)) = \log_{10}(C(0)) - \frac{t}{D(T, pH)} \quad (2)$$

where  $C(0)$  (CFU/g) is the initial population at time 0 (min),  $t$  (min) is the processing time,  $C(t)$  (CFU/g) is the population at time  $t$ , and  $D(T, pH)$  (min) is the decimal reduction time at temperature  $T$  (°C) and pH at time of treatment.

The secondary model is an extension of the Bigelow model (Couvert et al., 2005). In addition to the effect of a temperature change on  $D$ , this model also describes the effect on  $D$  of a pH change. It is expressed as

$$D(T, pH) = D_{ref} \cdot 10^{(T_{ref}-T)/z_T} \cdot 10^{(pH-pH_{ref})/z_{pH}} \quad (3)$$

where  $T$  (°C) is the temperature and  $pH$  is the pH of the product during processing,  $T_{ref}$  (°C) is the reference temperature, here fixed at the reference sterilization temperature of 121.1 °C,  $pH_{ref}$  (pH unit) is the pH of

reference, fixed at 7.0,  $z_T$  (°C) is the increase in temperature resulting in a ten-fold reduction in  $D$ ,  $z_{pH}$  (pH unit) is the decrease in pH resulting in a ten-fold reduction in  $D$ , and  $D_{ref} = D_{121.1^\circ C, pH7}$  (min) is the decimal reduction time at 121.1 °C and pH 7.

The heat resistance parameters  $D_{ref}$ ,  $z_T$  and  $z_{pH}$  were estimated from a meta-analysis performed on 430  $D$  values mainly issued from literature, using a hierarchical Bayesian model (Rigaux et al., 2013).  $D_{ref}$ ,  $z_T$  and  $z_{pH}$  were considered as both uncertain and variable parameters (Table 2). Correlations between the hyperparameters (mean and standard deviation (SD) of  $D_{ref}$ ,  $z_T$  and  $z_{pH}$ ) (Table 3) were taken into account in the simulation using the Iman and Conover method (Iman and Conover, 1982).

Blanching duration and time-temperature profiles during the sterilization of canned green beans were communicated by the company and considered as variable parameters as done by Pouillot et al. (2007) (Table 2). The temperature was considered as homogeneous in the canned green beans under the assumption of convective heat diffusion.

The product pH during blanching and sterilization was considered variable. The pH distributions were based on specific measurements (Section 2.3) and assumed to be normal with mean 6.4 and standard deviation (SD) 0.2 (respectively mean 5.8 and SD 0.2), truncated on [4.8; 7] (see Table 2).

### 2.5.3. Calculation of a sterilization equivalent time

A sterilization equivalent time as a function of the sterilization value  $F_0$  (the reference descriptor of the applied heat treatment determined with  $z_T = 10$  °C) and of the resistance parameter  $z_T$  of the specific target bacterium (here *G. stearothermophilus*) was built. Six sterilization time-temperature profiles communicated by the company were first converted into equivalent heat treatments of duration  $t = t_{eq}(z_T)$  at the target temperature  $T_{ref} = 121.1$  °C using Eq. (4) (Fernandez and Peck, 1997) as follows:

$$t_{eq}(z_T) = \sum_{i=1}^n (t_i - t_{i-1}) 10^{\frac{1}{z_T} \left( \frac{T_i + T_{i-1}}{2} - T_{ref} \right)} \quad (4)$$

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**Table 2**  
 Model parameters: description, variability and uncertainty distribution, mean and 95% CI of the global marginal distribution.

Parameter	Description (unit)	Variability distribution	Uncertainty distribution	Mean and 95% probability range <sup>a</sup>	Source <sup>b</sup>		
Contamination param.	$CATS_{mit}$	ATS concentrations in fresh green beans ( $\log_{10}CFU/g$ )	$N(M_{CATSi}; S_{CATSi})$	$M_{CATSi} \sim N(0.44; 0.24)$ $S_{CATSi} \sim LnN(-0.11; 0.39)$	0.4 [-1.7; 2.6]	Spec. data	
	$CATS_{blwater}$	ATS concentrations in blanching water ( $\log_{10}CFU/g$ )	$N(M_{CATSbw}; S_{CATSbw})$	$M_{CATSbw} \sim N(2.26; 0.18)$ $S_{CATSbw} \sim LnN(0.17; 0.10)$	2.3 [-0.1; 4.6]	Spec. data	
	$CATS_{brine}$	ATS concentrations in brine ( $\log_{10}CFU/g$ )	$N(M_{CATSbr}; S_{CATSbr})$	$M_{CATSbr} \sim N(0.37; 0.14)$ $S_{CATSbr} \sim LnN(0.13; 0.07)$	0.4 [-1.9; 2.6]	Spec. data	
	$ratio_{mit}$	Proportion of Gbs among ATS in fresh green beans or in brine	-	$B(78; 167)$	0.32 [0.26; 0.38]	Spec. data	
	$ratio_{blwater}$	Proportion of Gbs among ATS in blanching water	-	$B(72; 39)$	0.65 [0.56; 0.73]	Spec. data	
	$\rho_{soak}$	Water soak rate at blanching	-	$BP(1\%; 2.7\% 6\%)$	0.03 [0.01; 0.05]	Spec. data	
Microbial param. of <i>G. stearothermophilus</i>	$D_{ref}$	Time (min) to the first decimal reduction at $T = 121.1$ °C and $pH = 7$	$10^{N(M_{LD}, S_{LD})}T(0.1; 30)$	$M_{LD} \sim N(0.432; 0.024)^c$ $S_{LD} \sim LnN(-1.277; 0.073)^c$	3.3 [0.8; 9.6]	Rigaux et al. (2013)	
	$Z_T$	Increase in temperature resulting in a ten-fold reduction in $D_{ref}$ (°C)	$N(M_{ZT}, S_{ZT})T(3; 15)$	$M_{ZT} \sim N(9.13; 0.34)^c$ $S_{ZT} \sim LnN(0.60; 0.19)^c$	9.1 [5.5; 12.9]	Rigaux et al. (2013)	
	$Z_{pH}$	Decrease in pH resulting in a tenfold reduction in $D_{ref}$ (pH unit)	$N(M_{ZpH}, S_{ZpH})T(1; 13)$	$M_{ZpH} \sim LnN(1.45; 0.09)^c$ $S_{ZpH} \sim \Gamma(1.99, 3.29)^c$	4.3 [2.9; 6.2]	Rigaux et al. (2013)	
	$\rho_{germ}$	Spore germination probability	-	$B(4.6; 1.4)$	0.8 [0.4; 1.0]	Expert op.	
	$C_{max}$	Maximum bacterial concentration in the product ( $\log_{10}CFU/g$ )	-	$N(9.00; 0.25)$	9.0 [8.5; 9.5]	Expert op.	
	$\mu_{opt}$	Optimal growth rate ( $h^{-1}$ )	$BP(2.01; ML_{\mu}; 2.72)$	$ML_{\mu} \sim BP(2.20; 2.31; 2.43)$ $N(5; 0.1)$	2.3 [2.1; 2.6]	Based on Llaudes et al. (2001)	
	$pH_{min}$	Minimal growth cardinal pH	-	$N(5; 0.1)$	5.0 [4.8; 5.2]	Expert op.	
	$pH_{opt}$	Optimal growth cardinal pH	-	$N(7; 0.1)$	7.0 [6.8; 7.2]	Expert op.	
	Environmental param.	$pH_{blan}$	Green bean pH during blanching	$N(6.4; 0.2)T(4.8; 7)$	-	6.4 [6.0; 6.8]	Spec. data
		$pH_{ster}$	Green bean pH during sterilization	$N(5.8; 0.2)T(4.8; 7)$	-	5.8 [5.4; 6.2]	Spec. data
$pH_{FP}$		Final sterilized product pH	$N(5.55; 0.1)T(4.8; 7)$	-	5.5 [5.3; 5.7]	Spec. data	
$t_{blan}$		Blanching duration (min)	$BP(3.5; 6; 10)$	-	6.3 [4.2; 8.7]	Company	
$T_{blan}$		Blanching temperature (°C)	$BP(85; 91; 98)$	-	91.1 [86.7; 95.8]	Company	
$F_0$		Value of sterilization (min)	$LnN(3.40, 0.11)$	-	30.2 [24.3; 37.2]	Company + model	

Notes:  $N(a; b)$  (resp.  $N(a; b)T(c, d)$ ,  $LnN(a; b)$ ) stands for the normal distribution (resp. the normal distribution truncated on  $[c; d]$ , the lognormal (base e) distribution based on a normal distribution) of mean  $a$  and standard deviation  $b$ .  $\Gamma(a; b)$  stands for the gamma distribution with shape parameter  $a$  and rate parameter  $b$ .  $BP(a; b; c)$  stands for a BetaPert distribution of minimum value  $a$ , most likely value  $b$  and maximum value  $c$ .  $B(a; b)$  stands for a Beta distribution of parameters  $a$  and  $b$ .

<sup>a</sup> Values are based on the confounded analysis of variability and uncertainty and were determined by Monte Carlo simulation (see Section 2.2).

<sup>b</sup> "Spec. data", data specifically collected for the present work; "Expert op.", expert opinion.

<sup>c</sup> Some correlations between the hyperparameters (mean and SD) of  $D_{ref}$ ,  $Z_T$  and  $Z_{pH}$  were previously observed (Rigaux et al., 2013), and are defined in Table 3. They may be taken into account using the method of Iman and Conover (1982), which is implemented in the R package mc2d (Pouillot and Delignette-Muller, 2010).

where  $t_i$  (min) and  $T_i$  (°C) are the observed time and temperature at the  $i$ th measurement point of a time-temperature profile of  $n$  points  $(t_i, T_i)_{i=1, \dots, n}$ .

The resulting equivalent time  $t_{eq}(Z_T)$  depends on *G. stearothermophilus*  $Z_T$ , which varies from can to can at each iteration according to its probability distributions (Fig. 2a, Table 2). A conversion model accounting for the dependence between  $Z_T$  and  $t_{eq}$  and separating the effect of the bacteria resistance and of the heat treatment was built as follows. An almost linear dependence (Fig. 2.b,  $R^2 = 0.88$ ) was observed between  $\ln(t_{eq})$  and  $1/Z_T$  (which can be explained by the very low values, and consequently the non-significance, of the terms obtained in Eq. (4) at temperature <100 °C). Then, following the envelope method (Vose, 2000), a least square regression of  $\ln(t_{eq})$  on  $1/Z_T$  was performed and

modeled by:

$$\ln(t_{eq}) = a + b\left(\frac{1}{Z_T}\right) + \varepsilon \quad (5)$$

$\varepsilon \sim N(0, c)$  stands for a normal distribution of mean 0 and SD  $c$  and is the model error, which here mainly represents the variation in the applied time-temperature profiles. The homogeneity of the error variance was assumed to simplify the model and to separate the effect of the heat treatment intensity ( $F_0$ ) and that of  $Z_T$  (describing the effect of temperature changes on microbial resistance).

Eq. (5) was then reformulated into Eq. (6) (Fig. 2.b):

$$t_{eq} = \exp\left(b\left(\frac{1}{Z_T} - \frac{1}{Z_{TREF}}\right)\right) \cdot \exp(X) \quad (6)$$

where  $X = a + \frac{b}{Z_{TREF}} + \varepsilon \sim N\left(a + \frac{b}{Z_{TREF}}, c\right)$ .

By definition,  $F_0$  corresponds to the sterilization equivalent time at 121.1 °C determined with reference value  $Z_T = Z_{TREF} = 10$  °C. From Eq. (6)  $t_{eq} = \exp(X)$  when  $Z_T = Z_{TREF} = 10$  °C. Thus  $F_0 = \exp(X)$  in Eq. (6) and Eq. (6) becomes Eq. (7):

$$t_{eq}(Z_T, F_0) = \exp\left(b\left(\frac{1}{Z_T} - \frac{1}{Z_{TREF}}\right)\right) \cdot F_0. \quad (7)$$

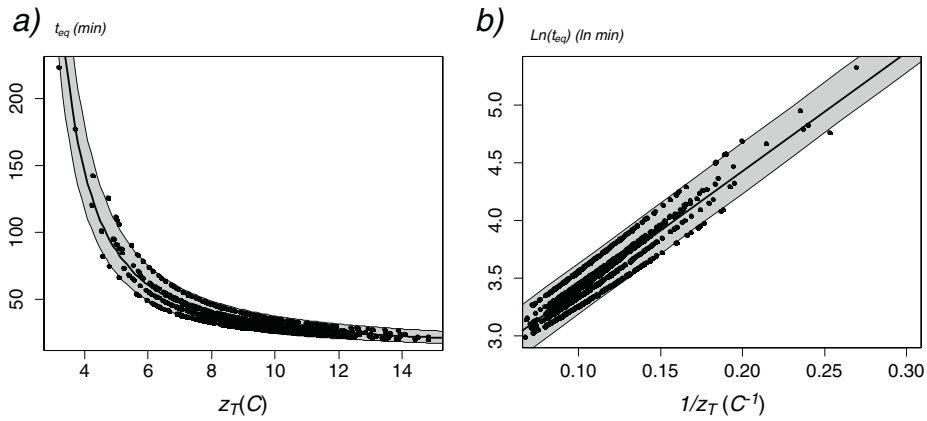
The resulting  $F_0$  variability distribution is a lognormal distribution based on a normal distribution of mean 3.40 and standard deviation of

**Table 3**  
 Spearman rank correlation between the hyperparameters of *G. stearothermophilus* heat resistance parameters  $D_{ref}$ ,  $Z_T$  and  $Z_{pH}$  (Rigaux et al., 2013). Definitions of hyperparameters may be seen in Table 2.

Correlations	$M_{ZpH}$	$S_{ZpH}$	$M_{ZT}$	$S_{ZT}$	$M_{LD}$	$S_{LD}$
$M_{ZpH}$	1	0.70	0.01	0.01	-0.11	-0.06
$S_{ZpH}$	0.70	1	0.00	0.01	-0.07	-0.03
$M_{ZT}$	0.01	0.00	1	0.62	0.36	-0.39
$S_{ZT}$	0.01	0.01	0.62	1	0.33	-0.49
$M_{LD}$	-0.11	-0.07	0.36	0.33	1	-0.21
$S_{LD}$	-0.06	-0.03	-0.39	-0.49	-0.21	1

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**Fig. 2.** Sterilization equivalent time at 121.1 °C  $t_{eq}$  as a function of the heat resistance parameter  $z_T$  (a),  $Ln(t_{eq})$  as a function of  $1/z_T$  (b), and envelopes of the adjusted models. Points correspond to a sample of 1000  $z_T$  (or  $1/z_T$ ) values and their corresponding equivalent times  $t_{eq}$  (or  $Ln(t_{eq})$ ) determined from Eq. (4). The bold middle lines represent the adjusted models (Eqs. (7) and (5)), and the bounding lines represent the 95% CI of the envelope (colored in gray).  $t_{eq}$  (or  $Ln(t_{eq})$ ) values corresponding to points in the envelope with a fixed  $z_T$  (or  $1/z_T$ ) value represent the 95% variability interval of the heat treatment.

0.11 (corresponding to estimations  $\hat{a} = 2.36 \ln(\text{min})$ ,  $\hat{b} = 10.41 \text{ } ^\circ\text{C}$  and  $\hat{c} = 0.11 \ln(\text{min})$ ), leading to  $F_0$  values of mean 30.2 min and 95% variability interval [24.1, 37.2]min, which is fully consistent with the  $F_0$  determined by the company from the time-temperature profiles.

#### 2.5.4. Cross-contamination models

Cross-contamination was simply assumed to bring a quantity of new spores in the can, following Eq. (8):

$$N_X = N_{X-1} + N_{Xcc} \quad (8)$$

where  $N_X$  (resp.  $N_{X-1}$ ) (CFU) is the quantity of *G. stearothermophilus* at the food chain step  $X$  (resp. at the step before step  $X$ ) and  $N_{Xcc}$  is the quantity of *G. stearothermophilus* brought by cross-contamination at step  $X$ .

The first source of cross-contamination is the blanching water. The quantity of spore contamination from the blanching water was considered as proportional to the amount of water impregnating green beans during this process step. The impregnating rate  $\rho_{soak}$  was estimated at around 2.7% using data obtained from an experimental measurement in laboratory consisting in weighing some green bean samples before and after a cooking process (data not shown)(see Table 2 for the adjusted distribution). The quantity of spores added (CFU) by cross-contamination at blanching is then defined by

$$N_{BLANcc} = w_{GB} \cdot \rho_{soak} \cdot C_{blwater} \quad (9)$$

where  $w_{GB} = 445 \text{ g}$  is the green bean weight at blanching, and  $C_{blwater}$  (CFU/g) is the *G. stearothermophilus* concentration in blanching water.

The second source of contamination comes from the recovery liquid at filling. The number of spores added (CFU) by cross-contamination from the recovery liquid is simply defined by

$$N_{BRINEcc} = w_{brine} \cdot C_{brine} \quad (10)$$

where  $w_{brine} = 405 \text{ g}$  is the brine weight in a can and  $C_{brine}$  (CFU/g) is the *G. stearothermophilus* concentration in brine defined in Section 2.2.

$N_X$ ,  $N_{X-1}$ ,  $N_{Xcc}$ ,  $N_{BLANcc}$  and  $N_{BRINEcc}$  are described with continuous distributions.

#### 2.5.5. Incubation model

The incubation module is made of 3 steps: germination of spores, growth of vegetative cells and non-stability. Germination of spores may occur after sterilization. Each spore was assumed to have a

probability  $p_{germ}$  of germinating. The model is defined by Eq. (11):

$$N_{Germination} = \text{Bin}(N_{STERI}, p_{germ}) \quad (11)$$

where  $\text{Bin}(a,b)$  stands for a binomial distribution of parameters  $a$  and  $b$ , and  $N_X$  (CFU) is the (rounded down to the next integer) number of spores at step  $X$ .

The primary growth model is the logistic model with delay (Rosso et al., 1995), defined by:

$$C(t) = \begin{cases} C(0) & \text{if } t < \lambda \\ C_{max} / \left[ 1 + \left( \frac{C_{max}}{C_0} - 1 \right) \exp(-\mu_{max}(t-\lambda)) \right] & \text{if } t \geq \lambda \end{cases} \quad (12)$$

where  $t$  (h) is the duration of the growth step,  $C(0)$  and  $C(t)$  (CFU/g) are respectively the modeled concentrations at time  $t = 0$  and at time  $t$ ,  $C_{max}$  (CFU/g) is the maximum bacterial concentration in the product,  $\mu_{max}$  ( $\text{h}^{-1}$ ) the maximum growth rate and  $\lambda$  (h) the lag time.  $\lambda = 0$  was assumed in this work as growth occurs in rather optimal conditions and as a fail-safe assumption.

The secondary growth model is based on the ‘‘Gamma’’ model developed by Zwietering et al. (1992), and describes  $\mu_{max}$  as a function of environmental conditions such as temperature, pH and water activity:

$$\mu_{max} = \mu_{opt} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \quad (13)$$

where  $\mu_{opt}$  ( $\text{h}^{-1}$ ) is the optimal growth rate in the product,  $\gamma(T)$ ,  $\gamma(pH)$  and  $\gamma(a_w)$  describe the effect of the temperature  $T$ , the pH and the water activity  $a_w$  on the growth rate. As water activity in green beans and the incubation temperature ( $T = 55 \text{ } ^\circ\text{C}$ ) are almost optimal for the growth of *G. stearothermophilus*, it was assumed  $\gamma(T) = 1$  and  $\gamma(a_w) = 1$ . The pH effect is described by Rosso et al. (1995):

$$\gamma(pH) = \frac{(pH - pH_{min})(pH + pH_{min} - 2 pH_{opt})}{(pH - pH_{min})(pH + pH_{min} - 2 pH_{opt}) - (pH - pH_{opt})^2} \quad (14)$$

where  $pH_{min}$  and  $pH_{opt}$  (pH unit) are the minimal and optimal cardinal pH of *G. stearothermophilus*, and where  $pH_{max}$  in the original model was considered as equal to  $2 \cdot pH_{opt} - pH_{min}$ . (Augustin and Carlier, 2000).

Changes in pH were detected in food or medium containing populations higher than  $10^7 \text{ G. stearothermophilus ml}^{-1}$  (Llaudes et al., 2001; Yoo et al., 2006), and within 24 h incubation at  $55 \text{ } ^\circ\text{C}$  (Llaudes et al., 2001). Non-stability was therefore assumed to occur if

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*G. stearothermophilus* concentration exceeds  $7 \log_{10}$ CFU/g before 72 h incubation at 55 °C. This assumption is rather conservative, as time to spoilage accounted in the model (at least 72 h) is substantially longer than the one reported in these previous works (usually 24 h).

The probability distributions on all the parameters used in the germination and growth models are described in Table 2. Specific laboratory experiments to determine the growth rate of spores of *G. stearothermophilus* strains (CTCPA 2804 173, 2804 138, 2804 168) were produced as previously described (André et al., 2012) and separately spiked at 10 spores/g and  $10^2$  spores/g in 50 g of green beans in 50 ml of brine. Despite suboptimal pH,  $\mu_{max}$  was of the same order of magnitude as that observed in other media and foods (Heinrich et al., 2008; Ng and Schaffner, 1997; Ng et al., 2002; Thompson and Thames, 1967; Yoo et al., 2006). We therefore assumed that canned green beans were a highly suitable medium for *G. stearothermophilus* growth and set the most likely value of  $\mu_{opt}$  at  $2.3 \text{ h}^{-1}$ , based on the cell doubling time  $T_d$  reported by Llaudes et al. (2001) in tryptic soy broth ( $\mu_{opt} = \ln(2) / T_d$ ). As food conditions were highly suitable for growth and in absence of specific data, a high germination was also hypothesized and the probability of germination  $p_{germ}$  was set at around 90%. The pH of the sterilized product in which growth will occur was estimated at 5.5 pH unit on average, based on specific measurements (Section 2.3). *G. stearothermophilus*  $C_{max}$  values are between  $10^7$  and  $10^{11}$  (Thompson and Thames, 1967; Ng and Schaffner, 1997; Yoo et al., 2006; Heinrich et al., 2008; André unpublished data 2011). Mean  $C_{max}$  was taken at  $10^9$ , which is the middle of the range (on a  $\log_{10}$  scale) of these previously reported values.  $pH_{opt}$  was set at 7.0, which agrees with the optimal growth pH of *G. stearothermophilus* at pH 6.2–7.5 (Logan and De Vos, 2009).  $pH_{min}$  was set at 5.0: no growth at pH 5.0 in green beans and possible growth at pH 5.2 have been reported in diverse instances (André, CTCPA, unpublished data).

## 2.6. Sensitivity analysis

Sobol sensitivity indices were determined by the Saltelli variance-based method (Saltelli, 2002) and computed with the R package sensitivity (Pujol et al., 2012). This method has been selected for its model independence, its capacity of integrating non-linearity, thresholds and interactions, and of treating untransformed variables (no need of discretization) (Frey et al., 2003; Ellouze et al., 2010).

The sensitivity analysis was performed on the variability parameters from samples of size  $N_v = 100,000$  (drawn from their variability distributions conditionally to a set of uncertain values), and was independently repeated for  $N_u = 10,000$  realizations of uncertainty, leading to an uncertainty distribution on the sensitivity indices. This approach, initially developed in association with an ANOVA by Mokhtari and Frey (2005) and further applied by Pouillot et al. (2007) and Membré et al. (2008), was here used with a computation of Sobol indices. The model response was *G. stearothermophilus* concentration after sterilization,  $C_{STERI}$  (in  $\log_{10}$ CFU/g). To focus on the most relevant factors and for computational ease, the *G. stearothermophilus* concentrations  $C_x$  were used instead of factors  $CATS_x$  and  $ratio_x$  (see Eq. (1), in Section 2.5.1). The validity of the Monte-Carlo estimation of the sensitivity indices was guaranteed by the independence of all factors (Saltelli, 2002). Sobol indices are comprised between 0 and 1. The highest Sobol indices indicate factors having the highest influence on the variability of the model response.

## 2.7. What-if scenarios

What-if scenarios were explored to test the effect of some decisions on the management of the risk of spoilage (scenarios 1 to 12) or of model assumptions (scenarios 13 to 16) on the estimated % of non-stability (Table 4). In scenarios 1 to 3, different  $F_0$  values were explored in the range [10; 25] min to test the effect of a decrease in the heat treatment at sterilization (mean  $F_0$  in the reference model = 30.3 min). In

scenarios 4 and 5, some better hygiene measures were tested, assuming no cross-contamination at the blanching or brining step, in combination with different  $F_0$  values (scenarios 6 and 7). Poor hygiene scenarios corresponding to higher microbial loads at the blanching and/or brining steps were explored (scenarios 8 to 10). The effect of a pH drop, obtained for instance by organic acid addition at brining as applied on some canned vegetables, was tested (scenarios 11 and 12). Scenarios 13 to 15 tested the impact of different germination rates. Finally, the effect of assuming independence between the hyperparameters of  $D_{ref}$ ,  $z_T$  and  $z_{pH}$  (see Section 2.5.2 and Tables 2 and 3) was tested in scenario 16. The simulations were performed with  $N_u = 1500$  and  $N_v = 100,000$ .

## 3. Results

### 3.1. Fitted distributions of *G. stearothermophilus* from microbiological analyses at several processing steps

The microbiological analyses performed at several processing steps (Section 2.2) consistently showed the presence of significant populations of spores of aerobic thermophilic bacteria. Their statistical distributions are shown and designated as “contamination parameters” in Table 2. Among those, a high percentage of *G. stearothermophilus* (often  $\geq 30\%$  of the ATS) was detected in the surveyed samples. For instance, the concentration of *G. stearothermophilus* spores in raw unprocessed green beans was estimated at a mean of  $-0.1 \log_{10}$ CFU/g with a 95% variation range  $[Q_{0.025}, Q_{0.975}] = [-1.9, 1.7] \log_{10}$ CFU/g (Table 5).

### 3.2. Predicted changes in concentrations during processing and non-stability prevalence

Two processing steps have a major impact on changes in the concentrations in *G. stearothermophilus* (Fig. 3). These are the blanching step, which results in a global increase in the bacteria concentration due to cross contamination (the inactivation during this process operation is negligible:  $-1\%$  on average), and the sterilization, which results in a high microbial inactivation. Clear-cut situations were observed during the 7-day incubation at 55 °C: either growth leading to spoilage or no growth at all.

**Table 4**

What-if-scenarios and corresponding non-stability rates (estimation[95% CI]). Parameters are described in Table 2 or in Section 2.5.

	What-if-scenario	Non-stability rate (%)
0	Reference model	0.5 [0.1; 1.2]
1	$F_0 = 25$ min	0.9 [0.3; 2.0]
2	$F_0 = 20$ min	2.0 [0.8; 3.9]
3	$F_0 = 10$ min	13.5 [8.5; 20.0]
4	$N_{BLANcc} = 0$ CFU	0.2 [0.0; 0.7]
5	$N_{BRINEcc} = 0$ CFU	0.4 [0.1; 1.0]
6	$F_0 = 20$ min and $N_{BLANcc} = 0$ CFU	1.1 [0.4; 2.6]
7	$F_0 = 25$ min and $N_{BLANcc} = 0$ CFU	0.5 [0.1; 1.3]
8	$CATS_{bWater} \sim N(4.3; 1.2) \log_w$ CFU/g	1.6 [0.7; 3.0]
9	$CATS_{bme} \sim N(2.4; 1.1) \log_w$ CFU/g	1.1 [0.4; 2.1]
10	$CATS_{bWater} \sim N(4.3; 1.2) \log_{10}$ CFU/g and $CATS_{bme} \sim N(2.4; 1.1) \log_w$ CFU/g	1.9 [0.8; 3.5]
11	$pH_{steri} = 5.55$ and $pH_{FP} = 5.25$	0.2 [0.0; 0.7]
12	$pH_{steri} = 5.45$ and $pH_{FP} = 5.15$	0.1 [0.0; 0.5]
13	$p_{germ} = 0.90$	0.5 [0.1; 1.2]
14	$p_{germ} = 0.10$	0.2 [0.0; 0.7]
15	$p_{germ} = 0.01$	0.1 [0.0; 0.3]
16	Independence between the hyperparameters of $D_{ref}$ , $z_T$ and $z_{pH}$ (see Tables 2 and 3)	0.5 [0.1; 1.3]

The non-stability rates were evaluated on the variability dimension. The point estimate and the 95%CI were respectively provided through the 50th, 2.5th and 97.5th percentiles evaluated in the uncertainty dimension. See Pouillot et al. (2007) for details on the estimation of some statistics after a second-order Monte Carlo simulation.

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Table 5 gives statistics on the evolution of the *G. stearothermophilus* concentration, distinguishing uncertainty and variability in the results. Prevalence, which measures the proportion of cans containing at least one CFU is equal to 99.8% in unprocessed green beans, increases to 100.0% after blanching and decreases to 0.5% after sterilization. The variability of the concentrations in 95% of the cans is high before sterilization (e.g., 95% variation range =  $[-0.7; 3.0 \log_{10}\text{CFU/g}$  at blanching). After sterilization, 95.5% of cans do not contain any spore. In positive cans (i.e. cans containing at least one spore), the *G. stearothermophilus* concentration is quite variable (95% variation range =  $[-2.9; -0.3] \log_{10}\text{CFU/g}$ , which corresponds to 1 to 426 CFU per can) (Table 5 and Fig. 3). The uncertainty associated with these different estimations is relatively low, except for the concentrations in the positive cans after incubation (95% CI on the lower limit of the 95% variability interval of  $[2.9, 9.3] \log_{10}\text{CFU/g}$ ). The impact of variability is higher than the weight of uncertainty in the dispersion of concentrations just after sterilization (Fig. 3), justifying the interest of performing a sensitivity analysis with regard to variability sources.

The model predicts that about 92% of the cans containing at least one spore after sterilization will develop spoilage. This is favored by the high growth ability of germinated spores, since 99.9% of the cans containing at least one vegetative cell after spore germination will develop spoilage. Before 72 h and mostly around 24 h, *G. stearothermophilus* concentration in positive cans exceeds  $7 \log_{10}\text{CFU/g}$ , which corresponds to the minimal concentration required for spoilage (Fig. 4). Finally, the predicted non-stability rate is of 0.5% with a 95% uncertainty interval of  $[0.2\%; 1.2\%]$  and is equal to the prevalence rate after sterilization ( $0.5\%, 95\text{CI} = [0.2\%; 1.2\%]$ ).

### 3.3. Validation of the model with independent data

These predictions have been compared with two sets of observed data for model validation. These data were independent of the ones used for model construction. The first dataset consists in *G. stearothermophilus* concentrations in green beans immediately before can filling, defined as the product of the measured ATS concentration and the measured *G. stearothermophilus* ratio (see Section 2) (Fig. 3). This concentration ( $0.9 \log_{10}\text{CFU/g}$ ,  $95\% \text{CI} = [-0.1; 1.8]$ ) is equal to the modeled median concentration before filling ( $0.9 \log_{10}\text{CFU/g}$ ) and is totally included in the predicted 95% variation range ( $[-0.7; 3.0] \log_{10}\text{CFU/g}$ ), which is quite satisfying.

The second set of data compared with predictions is the percentage of non-stability at  $55^\circ\text{C}$  due to *G. stearothermophilus* reported by green bean processors. The general percentage of non-stability (AFNOR, 1997) recorded on a ten-year survey by 13 processors was equal to 1.5% with a 95% Clopper–Pearson proportion confidence interval (CPpCI) of  $[1.3\%; 1.6\%]$  for a total of approximately 63,000 tested green bean cans (André, CTCPA, unpublished data). The observed percentage of non-stability specifically due to *G. stearothermophilus* was obtained by multiplying the global percentage of non-stability by the percentage of non-

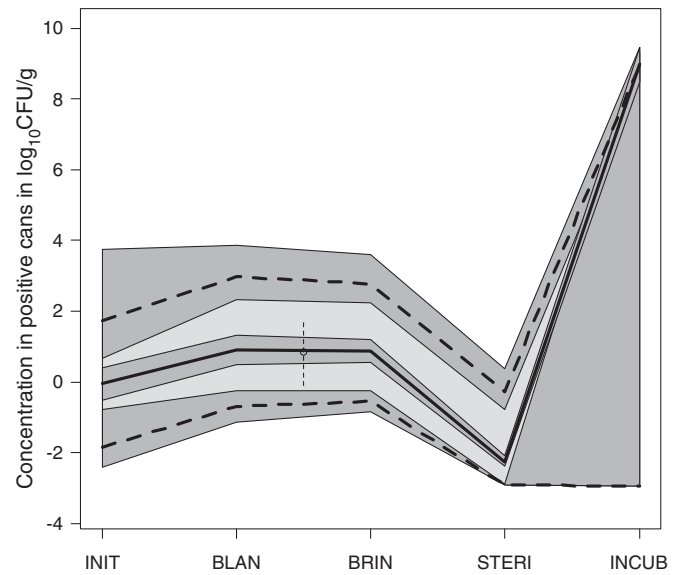


Fig. 3. *G. stearothermophilus* concentrations in positive cans modeled at the different steps X of the food chain (distinguishing uncertainty and variability). The solid thick line represents the median concentration, the dashed thick lines represent the limits of the 95% variability interval, and the dark gray zones represent the 95% uncertainty intervals on these statistics. The small circle represents the observed concentrations measured in green beans just before filling (see Section 2.2), and the vertical dashed segment represents the corresponding 95% CI.

stability specifically due to *G. stearothermophilus* in canned green beans. This latter percentage is approximately equal to 58% (with a 95% CPpCI of  $[33\%; 80\%]$ ) (André et al., 2013). Consequently, the general percentage of non-stability due to *G. stearothermophilus* was estimated at 0.9%, with a 95%CI of  $[0.5\%; 1.2\%]$ . The specific observed percentage of non-stability at  $55^\circ\text{C}$  of the 643 tested green bean cans at the period and in the plants where the microbiological analyses were performed was slightly less (not mentioned for confidentiality reasons) than the general percentage of non-stability. Consequently the specific observed percentage of non-stability specifically due to *G. stearothermophilus* was slightly less than 0.9%. This latter observed rate has to be compared with the modeled non-stability rate which was 0.5% ( $95\text{CI} = [0.1\%; 1.2\%]$ ). The two rates are very close and the credible intervals largely overlap.

### 3.4. Determination of the most influential factors by sensitivity analysis

The sensitivity analysis based on the calculation of Sobol indices (Table 6) detects the variable factors having a major influence on the variability of *G. stearothermophilus* concentration in green bean cans after sterilization. The variability range of each factor is the one defined in the model. The *G. stearothermophilus* heat resistance parameter  $D_{ref}$  is

Table 5

Statistics on changes along the food pathway in *G. stearothermophilus* prevalence and concentration in positive cans (i.e. containing at least one CFU), with separate uncertainty and variability. Estimation [95% CI] of prevalence, mean and median concentrations, and quantiles 0.025 and 0.975 of concentrations.

	Initial	Blanching	Brining	Sterilization	Incubation
Prevalence (%)	99.8 [91.8; 100.0]	100.0 [100.0; 100.0]	100.0 [100.0; 100.0]	0.5 [0.2; 1.2]	0.5 [0.2; 1.2]
$Q_{0.025}$ concentration ( $\log_{10}\text{CFU/g}$ )	-1.8 [-2.4; -0.8]	-0.7 [-1.1; -0.3]	-0.6 [-0.8; -0.3]	-2.9 [-2.9; -2.9]	-2.9 [-2.9; 9.3]
Median concentration	0.0	0.9	0.9	-2.3	9.0
$Q_{0.50}$ ( $\log_{10}\text{CFU/g}$ )	[-0.5; 0.4]	[0.5; 1.3]	[0.6; 1.2]	[-2.4; -2.1]	[8.5; 9.5]
Mean concentration ( $\log_{10}\text{CFU/g}$ )	0.9 [0.0; 3.9]	2.3 [1.6; 3.9]	2.1 [1.5; 3.6]	-1.0 [-1.6; 0.2]	9.0 [8.5; 9.4]
$Q_{0.975}$ concentration ( $\log_{10}\text{CFU/g}$ )	1.7 [0.7; 3.7]	3.0 [2.3; 3.9]	2.8 [2.2; 3.6]	-0.3 [-0.8; 0.4]	9.0 [8.5; 9.5]

All statistics were evaluated on the variability dimension. The point estimate and the 95% CI were respectively provided through the 50th, 2.5th and 97.5th percentiles evaluated in the uncertainty dimension.

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% of positive cans reaching  $7 \log_{10} \text{CFU/g}$

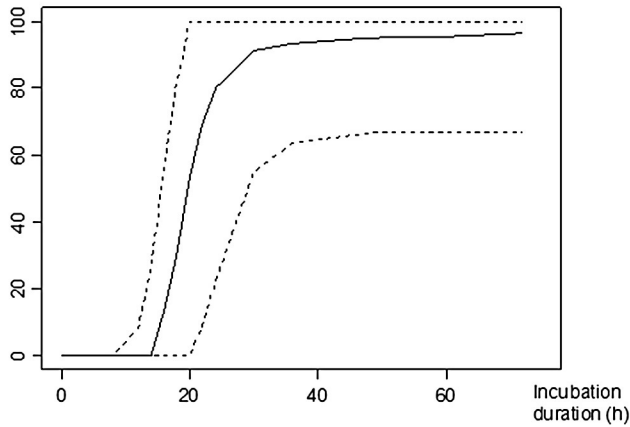


Fig. 4. Percentage of positive cans reaching  $7 \log_{10} \text{CFU/g}$  (corresponding to spoilage) as a function of the incubation duration. Solid lines represent the percentage estimation and dashed lines indicate the 95% CI, representing uncertainty around the estimation. The percentages were evaluated on the variability dimension. The point estimate and the 95% CI were respectively provided through the 50th, 2.5th and 97.5th percentiles evaluated in the uncertainty dimension.

the most influential factor: it explains 69% of the response variability ( $S_i = 0.69$  with  $95\% \text{CI} = [0.56; 0.79]$ ) and also has a high influence when interacting with other factors as shown by a high  $St_i$  at 0.91. The second most influential parameter is the *G. stearothermophilus* heat resistance parameter  $z_T$ . Its influence is linked to interactions with other factors as  $St_i$  (which accounts for interactions between factors) is equal to 0.18 and  $S_i$  is equal to only 0.04. The computation of second-order indices (data not shown) logically showed that the major interaction was between  $D_{ref}$  and  $z_T$  ( $S^2_{ij} \approx 0.10$ ). Four factors also have a significant (but much less pronounced) influence on the variability of the model response: the value of sterilization  $F_0$ , the product pH during sterilization  $pH_{steri}$ , the heat resistance parameter  $z_{pH}$  and the *G. stearothermophilus* contamination in blanching water  $C_{blwater}$  (respectively  $St_i = 0.05$ ,  $St_i = 0.05$ ,  $St_i = 0.03$  and  $St_i = 0.03$ ). The sources of uncertainty of the model parameters do not question the major influence of  $D_{ref}$  and  $z_T$  because the 95% CI on their  $St_i$  do not (or only marginally) overlap with other factors. However a noticeable exception is  $z_{pH}$ , with a large 95% CI on  $St_i$  suggesting a rather uncertain influence (from null to moderate) on the variability of the model.

In addition, the impact of different sources of uncertainty was analyzed in scenarios assuming no uncertainty on some parameters (or group of parameters). The highest sources of uncertainty are on

Table 6

Sensitivity analysis: estimation [95% CI] of the total effect Sobol indices  $St_i$ , and of the first-order Sobol indices  $S_i$ . The model output is the *G. stearothermophilus* concentration after sterilization,  $C_{STERI}$  (in  $\log_{10} \text{CFU/g}$ ).

Factors	$St_i$	$S_i$
$D_{ref}$	0.91 [0.83; 0.99]	0.69 [0.56; 0.79]
$z_T$	0.18 [0.10; 0.29]	0.04 [0.01; 0.09]
$F_0$	0.05 [0.04; 0.07]	0.01 [-0.01; 0.03]
$pH_{steri}$	0.05 [0.03; 0.08]	0.01 [-0.01; 0.03]
$z_{pH}$	0.03 [0.00; 0.16]	0.01 [-0.01; 0.04]
$C_{blwater}$	0.02 [0.01; 0.04]	0.01 [-0.01; 0.02]
$C_{brine}$	0.01 [0.00; 0.02]	0.00 [-0.01; 0.01]
$C_{INT}$	0.00 [0.00; 0.03]	0.00 [0.00; 0.01]
$t_{blan}$	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]
$T_{blan}$	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]
$pH_{blan}$	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]

Sobol indices were evaluated on the variability dimension. The point estimate and the indices 95%CI were respectively provided through the 50th, 2.5th and 97.5th percentiles evaluated in the uncertainty dimension.

the microbiological resistance parameters  $z_T$ ,  $z_{pH}$  and  $D_{ref}$ , and to a lesser extent the uncertainty on the different sources of (cross) contamination. The potential consequences of uncertainty were investigated with a sensibility analysis based on the calculus of Sobol indices in the confounded uncertainty and variability dimensions (parameters distributions reflecting both variations in uncertainty and variability). Even when including uncertainty, the parameters whose variation has the highest influence on *G. stearothermophilus* concentrations after sterilization are the same as those designated by the sensibility analysis in the variability dimension ( $D_{ref}$ ,  $z_T$ ,  $z_{pH}$ ,  $F_0$  and  $pH_{steri}$ ), but with a slightly higher influence of  $z_{pH}$  (results not shown).

### 3.5. What-if scenarios to test the influence of microbiological phenomena and options of spoilage risk management

Model predictions satisfactorily fitted with some observations (percentage of non-stability, concentrations at one processing step) (Section 3.2). Consequently the model can be used to test the consequences of some new scenarios not initially described by the model (Table 4).

The non-stability rate markedly decreases when the  $F_0$  increases, for instance with a non-stability rate of 13.5% for a  $F_0$  of 10 min and of 0.9% for a  $F_0$  of 25 min (Table 4, scenarios 1 to 3). Scenarios 4 and 5 show that assuming no cross-contamination during filling results in very little change in the response, whereas the impact of no cross-contamination at blanching results in a decrease in non-stability by half (medians of 0.2% versus 0.4%). Scenarios 6 to 7 show that improvement of hygiene (i.e. absence of cross-contamination at blanching) could allow a reduction of the sterilization value  $F_0$  with no or only a slight increase in the non-stability rate. Conversely, higher microbial loads at the blanching and/or the brining steps (hypotheses of a 2-log increase in ATS contaminations) caused up to four-times higher rates of non-stability (scenarios 8 to 10). A slight decrease in pH by 0.25 or 0.35 on average resulted in a marked decrease in the non-stability rates (scenarios 11 and 12). Scenarios 13 to 15 show that only a high decrease in the germination rate causes a significant decrease in the non-stability rate. The impact of assuming independence between the hyperparameters of the heat resistant parameters  $D_{ref}$ ,  $z_T$  and  $z_{pH}$  is also very low, with only a slight increase in the upper limit of the 95%CI representing uncertainty. However these results must be taken with caution because of the sometimes relatively high impact of uncertainty. For instance the uncertainty intervals of most non-stability rates overlap the uncertainty intervals of the reference model.

## 4. Discussion

The framework usually developed for the assessment of the microbial risk of foodborne poisoning was applied to the assessment of the risk of spoilage of canned food. The model estimated the changes in *G. stearothermophilus* concentration along a canned green-bean processing chain and gave a satisfactory prediction of the risk of non-stability reported by green bean canners. The predicted non-stability rate of 0.5%, with  $95\% \text{CI} = [0.2\%; 1.2\%]$  was close to the estimation of the industrial non-stability risk due to *G. stearothermophilus*, which was slightly less than 0.9%.

Consequently the model was considered suitable for testing the consequences of some risk management options or of some biological assumptions. Increasing the hygiene during processing (corresponding to the absence of *G. stearothermophilus* contamination at the blanching step) reduced the % of non-stability by half or kept it at the same level with a simultaneous reduction in the  $F_0$  value. Approximately the same reduction was obtained by an increase in  $F_0$  of 5 min in the range  $F_0 = 10$  to  $F_0 = 25$  min. A pH decrease could also interestingly reduce the percentage of non-stability. On the contrary, a poor respect of hygiene allowing high microbial loads in blanching water or in covering brine may lead to increase in non-stability rates. Moreover, the

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model hypothesized a high germination rate, meaning that most spores of *G. stearothermophilus* contaminating a can of green beans after sterilization would be able to germinate and grow. Germination and outgrowth of spores of *Bacillus* species and of *G. stearothermophilus* is generally impaired by heat stress, as shown by difficult recovery in suboptimal growth conditions (Feeherry et al., 1987; Leguerinel et al., 2005). That is why the consequences of different germination assumptions were tested. Current scientific knowledge does not suggest a very low germination rate of surviving spores after heat treatment. Using a more sophisticated model accounting for an interaction between heat shock intensity and probability of germination is therefore not necessary. The influence of the growth parameters could also have been tested, as a few data are currently available. However these data consistently show a rapid growth of *G. stearothermophilus* in foods and media at a range of pH similar to the one considered in this work, followed by a rapid spoilage (pH decrease).

Additionally, the model could be improved by new data and/or scientific knowledge. A possible additional cross-contamination source is the release of green bean debris during transfer along the food production chain. These debris have a much higher ATS concentration (mean at  $1.7 \log_{10}$ CFU/g, with a 95% variation range =  $[-2.0, 5.4]$  according to experimental measurements) than do fresh unprocessed green beans (Table 2). They sporadically cross-contaminate cans, but the rate of cross-contamination (% of contaminated cans and % of debris in those cans) is unknown. Consequently this was not integrated into the model. The primary inactivation model was assumed to be log-linear, although shoulders or tails are sometimes observed on survival curves; this is the most common assumption in the food industry (Rigaux et al., 2013). The absence of a selective medium for *G. stearothermophilus* led to dealing with ATS concentrations and confirmation tests.

Changes in *G. stearothermophilus* concentrations, sensitivity analyses and what-if-scenarios give consistent results. Important steps and risk factors were identified. The sterilization process is crucial (Fig. 3, Tables 4 and 6), resulting in a high spore inactivation. The factors implicated in the sterilization model are unsurprisingly the most influential factors according to the sensitivity analysis ( $D_{ref}$ ,  $Z_T$ ,  $pH_{steri}$ ,  $F_0$ ,  $Z_{pH}$ ) (Table 6). The *G. stearothermophilus* decimal reduction time  $D_{ref}$  is actually so influential that it explains, alone or in interaction with the other factors, 91% of the variability of the *G. stearothermophilus* concentration after sterilization. The second and the fifth most influential factors are also heat resistance parameters of *G. stearothermophilus* ( $Z_T$  and  $Z_{pH}$ ). These parameters cannot be modified, which consequently restricts possible management actions to limit spoilage risk. The fourth most influential factor,  $pH_{steri}$ , would also be difficult to modify. Nevertheless, the  $F_0$ , as well as the cross-contamination from blanching water ( $C_{blwater}$ ), appears to be quite influential and constitutes possible points of management intervention (as illustrated in what-if-scenarios 1 to 7).

Sensitivity analysis is increasingly applied to risk assessment with one-dimension (Zwietering and Van Gerwen, 2000; Frey et al., 2003; Mokhtari and Frey, 2005; Ellouze et al., 2010) or two-dimension models (Pouillot et al., 2007; Membré et al., 2008; Mataragas et al., 2010; Busschaert et al., 2011). Sensitivity analysis or what-if-scenarios may be used to identify the highest sources of variability and of uncertainty in the final model. They can also be used early in model construction for prioritizing additional research, and improving or simplifying the model. For instance the sensitivity analysis showed in this work the importance of product pH during sterilization ( $pH_{steri}$ ). Further pH measurements were therefore performed. What-if-scenarios exploring the effect of modeling assumptions showed that a complex germination model was certainly unnecessary. Based on the results of the sensitivity analysis (Table 6), fixing some parameters of low influence, such as blanching time and temperature, could be a reasonable option.

Spoilage control and product safety in the canning industry are based on simple principles: a minimal heat-treatment for low-acid foods ( $F_0 = 3$  min, the “*botulinum* cook”) and an additional heat-

treatment to guarantee microbiological stability. Increasing the heat-treatment reduces spoilage occurrence and unsurprisingly the model predictions give a similar conclusion. However this is a costly operation in terms of energy and impact on product organoleptic quality and nutritional value. The model suggests to some extent that hygiene control, a top priority for many food industries, may also be efficient in vegetable canning for the control of process intensity. Testing the consequences of process modifications with models, such as the one developed in this work, is efficient method for industry managers to prepare important decisions.

## Acknowledgments

This work is a partial fulfillment of author C. Rigaux's PhD Thesis, has been supported by Agence Nationale de Recherche Paris, France under contract ANR-09-ALIA-014 (Ribenuit project), and is a contribution to the research activity of Unité Mixte Technologique Qualiveg. Thanks are due to Dr Olivier Couvert (LUBEM, Quimper, France) and to Jean-Baptiste Denis (INRA, Jouy-en-Josas, France) for kind communication of data and helpful scientific discussions. Thanks are also due to Rachel Galland (Bonduelle, France) and to other industrial partners for kindly providing technical information about the process.

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