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Quantitative microbial spoilage risk assessment of plant-based milk alternatives by *Geobacillus stearothermophilus* in Europe

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ABSTRACT

Geobacillus stearothermophilus is one of the predominant spoilers of UHT-treated food products, due to its extremely heat-resistant spores. However, the surviving spores should be exposed to temperature higher than their minimum growth temperature for a certain time to germinate and grow to spoilage levels. Considering the projected temperature increase due to climate change, the events of non-sterility during distribution and transportation are expected to escalate. Hence, the aim of this study was to build a quantitative microbial spoilage risk assessment (QMRSA) model to quantify the risk of spoilage of plant-based milk alternatives within Europe. The model consists of four main steps: 1. Initial contamination of raw materials 2. Heat inactivation of spores during UHT treatment 3. Partitioning 4. Germination and outgrowth of spores during distribution and storage. The risk of spoilage was defined as the probability of *G. stearothermophilus* to reach its maximum concentration ($N_{max} = 10^{7.5}$ CFU/mL) at the time of consumption. The assessment was performed for North (Poland) and South (Greece) Europe, and the risk of spoilage was estimated for the current climatic conditions and a climate change scenario. Based on the results, the risk of spoilage was negligible for the North European region, while the risk of spoilage in South Europe was 6.2×10^{-3} 95% CI (2.3×10^{-3};1.1×10^{-2}) under the current climatic conditions. The risk of spoilage was increased for both tested countries under climate change scenario; from zero to 1.0×10^{-4} in North Europe, risk multiplied 2 or 3 in South Europe depending on air conditioning implementation at consumer's place. Therefore, the heat treatment intensity and the use of insulated trucks during distribution were investigated as mitigation strategies and led to significant reduction of the risk. Overall, the QMRSA model developed in this study can support risk management decisions of these products by quantify the potential risk under current climatic conditions and climate change scenarios.

1. Introduction

Over recent years, consumers' demand for plant-based alternatives has increased worldwide due to behavioural changes and special dietary needs, such as lactose intolerance. This shift in the consumer preferences in Europe has already been captured, since plant-based consumption has increased by 49% in past two years and reached total sales values of €3.6 billion in 2020 (Nielsen, 2021). Following this tremendous need, the dairy industry has focused on developing many innovative plant-based milk alternatives (PBMA) originated from nuts, cereals and legumes, such as oat, soy and pea. While soil constitutes the environmental niche of spore-forming bacteria (Carlin, 2011), plant-based proteins used as raw material might be contaminated with various thermophilic bacilli. Plant-based milk alternatives are heat process products that usually undergo a commercial sterilisation by ultra-high temperature (UHT) (Sethi et al., 2016), which is primarily designed to eliminate spores of *Clostridium botulinum* (Jay et al., 2008; Membré and van Zuijlen, 2011). Even though this thermal process is quite severe, it has been proven insufficient in eliminating spores of thermophilic spore-forming bacteria that include extremely heat resistant endospores (Scheldeman et al., 2006). Hence, *Geobacillus stearothermophilus* constitutes a microorganism of concern for these specific products, due to its extreme heat resistance.

Despite the microbial contamination with *G. stearothermophilus* spores, heat processed plant-based milk alternatives are considered shelf-stable and therefore distributed and stored at ambient temperature. This is due to the fact that the surviving spores should be exposed to temperature higher than their minimum growth temperature for a

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certain time to germinate and grow to spoilage levels (André et al., 2013; Bevilacqua et al., 2009; Rigaux et al., 2014). Given that minimum temperature for growth of *G. stearothermophilus* is relatively high ($T_{min} > 33$ °C) (Kakagianni et al., 2016), current temperature conditions prevent the extensive growth of thermophilic bacilli, including *G. stearothermophilus*, and therefore ensure their microbiological stability (Misiou et al., 2021). Given the projection provided by the Intergovernmental Panel on Climate Change (IPCC), temperature is expected to be increased by 1.0–5.7 °C by the end of the 21st century (IPCC, 2021). Thus, the expected increase in global mean surface temperature may increase the risk of spoilage, especially in hot climate regions and temperate climates (Kakagianni and Koutsoumanis, 2018; Misiou et al., 2021; Misiou and Koutsoumanis, 2021).

In order to tackle the potentially increased events of non-sterility and support food risk management decisions, a quantitative microbial spoilage risk assessment (QMRSA) can be employed (Pujol et al., 2013; Membré and Boué, 2018; Koutsoumanis et al., 2021). To the best of our knowledge, this is the first attempt to quantify the risk of spoilage of plant-based milk alternatives by *Geobacillus stearothermophilus*. Hence, the aim of this study is to build a probabilistic model to quantify the risk of spoilage of PBMA within Europe. As much as possible, variability and uncertainty were separated in the model to make results more accurate but also to facilitate future decisions (Nauta, 2000; Thompson, 2002). Uncertainty corresponds to lack of knowledge while variability reflects true heterogeneity in population. In the context of QMRSA, the risk of spoilage is defined as the probability of rejecting a food product at the time of consumption due to spoilage (Koutsoumanis et al., 2021).

In the present model, the risk of spoilage was defined as the probability of *G. stearothermophilus* to reach the spoilage level at the time of consumption. The spoilage occurs when the concentration reaches the maximum concentration of *G. stearothermophilus* in PBMA and therefore it was set to $10^{7.5}$ CFU/mL (Misiou et al., 2021). Spoilage risks were estimated for the current climatic conditions and a climate change scenario during winter or summer seasons. The assessment was performed for two countries, namely Poland and Greece as a representative of the North and South region, respectively. In addition, the heat inactivation intensity and the transportation with insulated trucks were investigated as mitigation strategies to reduce the risk of spoilage.

2. Materials and methods

2.1. Model description

The model describes the spoilage risk of plant-based milk alternatives from raw materials up to the time of consumption. The model includes four main steps: 1. Initial contamination of raw materials 2. Heat inactivation of spores during UHT treatment 3. Partitioning 4. Germination and outgrowth of spores during distribution and storage.

The four steps were associated with four modules, presented in detail below, along with the input variables and the distributions used to build the risk model (Table 1). The probabilistic model inputs were built with uncertainty and variability to consider as much as possible lack of knowledge and true heterogeneity, respectively.

2.1.1. Module 1: Raw materials

The initial contamination level in the raw materials were obtained from the literature (NIZO, 2022). A total thermophilic bacteria load from 10 up to 10,000 CFU/g for the different tested plant isolated proteins, including oat, almond, pea and fava proteins was reported. However, from 39 tested samples (n), only 6 samples exceeded (s) the detection limit (NIZO, 2022).

Considering the low frequency and the high level of contamination, the prevalence in raw materials was described through a Bernoulli distribution with a Beta distribution capturing the uncertainty.

$$Bernoulli(1, prevalence) \tag{1}$$

$$prevalence\ Beta(s+1, n-s+1) \tag{2}$$

The microbial concentration in the raw materials ($N_{0_{rm}}$) was described through a Pert distribution for the variability dimension and a Uniform distribution to capture the uncertainty in minimum, most likely and maximum parameters as follows:

$$N_{0_{rm}}\ Pert(Min; Mostlikely; Max) \tag{3}$$

With:

$$Min\ Uniform(0; 1) \tag{4}$$

$$Mostlikely\ Uniform(1; 3) \tag{5}$$

$$Max\ Uniform(3; 5) \tag{6}$$

Considering a tank (V_t) of 1000 L, the initial number of spores in the tank (N_{0_t}) was estimated as follows:

$$N_{0_t} = N_{0_{rm}} \times V_t \tag{7}$$

2.1.2. Module 2: Heat treatment

The heat inactivation parameters (D-values) of *G. stearothermophilus* were obtained from the literature. More specifically, 566 D-values for several strains and matrixes were obtained from 37 studies (**Supplementary Data** Table 1). The obtained D-values were transformed using a $\log_{10}$ transformation and plotted against temperature (**Supplementary Data** Fig. 1).

A linear secondary model, was fitted into the $\log_{10}$D values to estimate the effect of heat temperature on the kinetic parameter D, as following:

$$log_{10}D = log_{10}D_{ref} + \frac{(T_{ref} - T)}{z} + \varepsilon_1 \tag{8}$$

$$\varepsilon_1\ Normal(0, sd_1) \tag{9}$$

Where:

T_{ref} is the reference temperature equals to 121 °C, T is the applied temperature during thermal treatment, D_{ref} (min) is the decimal reduction time at the reference temperature T_{ref} and z (°C) is the temperature increase required to reduce D-value by 90%. The error (ε_1) incorporated in the model was considered as mainly representative of variability due to the different strains included in the D-values dataset and possibly of some uncertainty due to the data collection process (although unlikely considering the significant amount of data and sources) (see **Supplementary Data** Table 1).

The number of spores surviving the UHT treatment (N_{HT}) was estimated using a Poisson distribution, as suggested by Nauta (2001) and recently used by Santos et al., 2020:

$$N_{HT}\ Poisson(N_{0_t} \times Pr_{sur}) \tag{10}$$

$$Pr_{sur} = 10^{\left(\frac{-t_{HT}}{D}\right)} \tag{11}$$

Where:

N_{0_t} is the number of spores present in the raw material per tank, as defined in **section 2.1.1** and Pr_{sur} is the probability for one spore to survive the heat treatment (Nauta, 2001) deduced from Eq. (8).

2.1.3. Module 3: Partitioning

The filling of the packs (in 250 ml) which is performed as the last stage of the UHT treatment, is a partitioning process following a Poisson distribution as suggested by (Nauta, 2005). The UHT-treated plant-based milk alternatives are aseptically packed into sterile containers and therefore no additional contamination was considered at this stage. The number of spores per pack (N_p) was estimated as follows:

Table 1

Inputs implemented in the risk model. Values and probabilistic distributions used to build the quantitative spoilage risk assessment (QMRSA) of plant-based milk alternatives by *Geobacillus stearothermophilus*. The values included in this table corresponded to the current climatic conditions (baseline scenario).

Description	Abbreviation	Unit	Inputs value	Implementation in the model	Source
Module 1: Raw materials					
Number of samples, Positive samples	n, s	–	$n = 39$ $s = 6$	Eq. (2) with V & U separated	NIZO, 2022
Microbial concentration in raw materials	$N_{0_{rm}}$	Log CFU/ mL	Pert (Min; Most likely; Max)	Eq. (3) with V & U separated	NIZO, 2022
Volume of the tank	V_t	L	1000	Eq. (7) fixed	Expert knowledge
Module 2: Heat-treatment					
Reference temperature	T_{ref}	°C	121	Eq. (8) fixed	Expert knowledge
Decimal reduction time reference	D_{ref}	min	2.34	Eq. (8) fixed	Deduce from fitting D-values (Sup. data)
Temperature resistance	z	°C	10.70	Eq. (8) fixed	Fitting D-values (Sup. data)
Heat inactivation secondary model error (Eq.1)	sd_1	Log min	0.35	Eq. (9) V	Fitting D-values (Sup. data)
Time of the treatment	t_{HT}	sec	Uniform (3;9)	Eq. (8) V	Expert knowledge
Temperature of the treatment	T_{HT}	°C	Uniform (130;150)	Eq. (8) V	Expert knowledge
Module 3: Partitioning					
Volume of product unit, a pack	V_p	ml	250	Eq. (12) fixed	Assumption
Module 4: Growth during distribution and storage					
Minimum temperature of growth	T_{min}	°C	33.76	Eq. (17) fixed	Kakagianni et al., 2016
Optimum temperature of growth	T_{opt}	°C	61.82	Eq. (17) fixed	Kakagianni et al., 2016
Maximum temperature of growth	T_{max}	°C	68.14	Eq. (17) fixed	Kakagianni et al., 2016
Secondary cardinal temperature model error	sd_2	–	0.003	Eq. (18) U	Kakagianni et al., 2016
Optimum growth rate	μ_{opt}	h^{-1}	Empirical distribution based on μ_{opt} data & bootstrap	Eq. (16) with V & U mixed	Misiou et al., 2021
Minimum pH of growth	pH_{min}	–	5.65	Eq. (19) fixed	Misiou et al., 2021
Optimum pH of growth	pH_{opt}	–	6.74	Eq. (19) fixed	Misiou et al., 2021
Maximum pH of growth	pH_{max}	–	8.71	Eq. (19) fixed	Misiou et al., 2021
pH of the product	pH	–	7.00	Eq. (19) fixed	Assumption
Secondary cardinal pH model error	sd_3	–	0.056	Eq. (20) U	Misiou et al., 2021
Temperature during distribution	T_{tr}	° C	Empirical distribution based on T dataset	Eq. (17) V	Data loggers Sup. data
Time during distribution	t_{tr}	h	Uniform (2;12)	Eq. (13) with V Eq. (21)	Assumption
Percentage of insulated trucks	P_{truck}	–	Uniform (0.1;0.5)	Eq. (14) with V & U separated	Assumption
Temperature of the product storage-retail	T_r	°C	Below 25	Eq. (17) fixed	Assumption
Temperature of the product storage during the day at consumer place	T_d	°C	Empirical distribution based on T dataset	Eq. (17) with V	www.wunderground.com
Temperature of the product storage during the night at consumer place	T_n	°C	Empirical distribution based on T dataset	Eq. (17) with V	www.wunderground.com
Time of the product night-consumer	t_n	days	60	Eq. (22) fixed	Assumption
Time of the product storage day-consumer	t_d	days	60	Eq. (23) fixed	Assumption

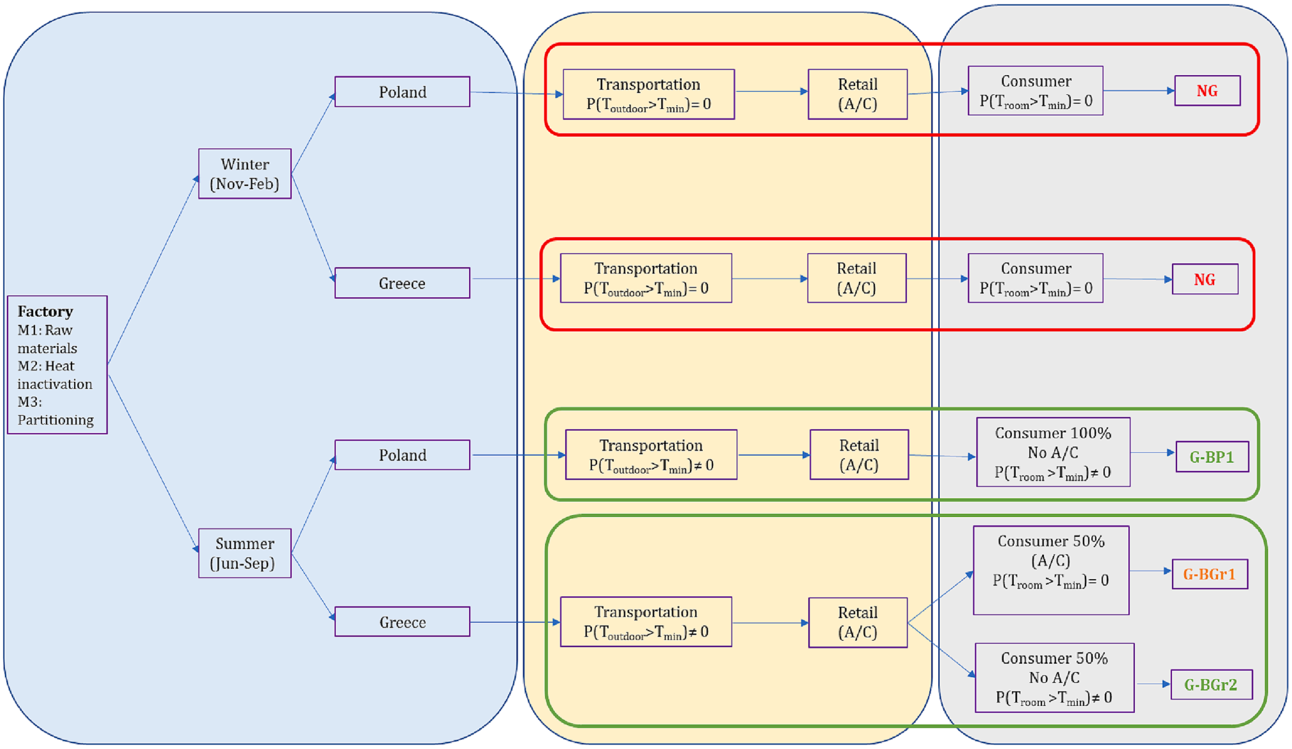


Fig. 1. Decision tree of the Quantitative Microbial Spoilage Risk Assessment (QMRSA) of plant-based milk alternatives by *Geobacillus stearothermophilus* in Europe. In the baseline scenario the production in Poland and Greece is split into two periods, namely winter and summer season. During winter season, no growth (NG) of *G. stearothermophilus* is observed since the probability of temperature during transportation and storage at retail and consumer stage exceeding the minimum temperature of growth equals to zero in both countries. During the summer season in Poland, a potential growth of *G. stearothermophilus* is assumed since temperature during transportation and storage at consumer stage may exceed the minimum temperature of growth (G-BP1). The latter is also valid for Greece (G-BGr2) except for the consumer stage for which it was assumed that half of the households have an air conditioning (A/C) system in place (G-BGr1).

$$N_p\ Poisson\left(\frac{N_{HT}}{V_t}*V_p\right) \tag{12}$$

2.1.4. Module 4: Growth during distribution and storage

In order to assess the risk of spoilage of plant-based milk alternatives distributed and stored within Europe, two representative countries were selected; namely Poland and Greece. The two selected countries were chosen to allow for a comparison between North and South Europe.

Since *Geobacillus stearothermophilus* is a thermophilic bacterium with a minimum temperature of growth around 33 °C, the following baseline scenario was designed to take into consideration the worst-case scenario based on the seasonality (see Fig. 1). In the baseline scenario, which corresponds to the current climatic conditions, the production in Poland and Greece was split into two periods, namely winter and summer season. During winter season, no growth (NG) of *G. stearothermophilus* was assumed, since temperature during transportation and storage at consumer stage remains below the minimum temperature of growth (33 °C) in both countries. Regarding summer production in Poland, a potential growth of *G. stearothermophilus* was assumed since temperature during transportation and storage at consumer stage may exceed the minimum temperature of growth (G-BP1). In the same vein, a potential growth was assumed for Greece during storage at consumer stage for the half of the households (G-BGr2). For the rest of the households, it was assumed that there is an air conditioning (A/C) system in place and therefore growth may only occur during transportation (G-BGr1). Since there are no available data, the above-mentioned assumptions were based on expert opinion. In all cases it was assumed that products were stored at retail stage below 25 °C and therefore no additional growth of *G. stearothermophilus* was occurred at this stage.

For the assessment of growth, the following assumptions were made. For the transportation time, it was assumed that based on the size of the tested countries, products were distributed from the factory to retailers for a period up to 12 h, the transportation time was thus described by a uniform distribution as follows:

$$t_{tr}\ Uniform(2;12) \tag{13}$$

Given that UHT products considered as shelf-stable, they can be transferred either by insulated or non-insulated trucks. Temperature in insulated trucks was assumed to remain below 25 °C (our best knowledge) and did not allow growth. The percentage of insulated truck was described through a Bernoulli distribution with Uniform distribution capturing the uncertainty as follows:

$$Bernoulli(1, P_{truck}) \tag{14}$$

$$P_{truck}\ Uniform(0.1;0.5) \tag{15}$$

Temperature inside non-insulated trucks was monitored through data loggers for a period of one month (August) during transportation in Greece, an empirical distribution was built using the recorded data. Due to lack of temperature data inside trucks in Poland, historical hourly outdoor temperature data for the same period were retrieved from an online database (www.wunderground.com) and used to build an empirical distribution.

The duration of the domestic storage was set to 120 days to take into account the worst-case scenario during summer period, while the temperature of domestic storage for both seasons was described by two probabilistic distributions build as follows. Historical hourly temperature data for Poland (Warsaw) and Greece (Athens) between June and September and November to February 2021 were retrieved from an online database (www.wunderground.com). To assess the variability, hourly temperature data for each country were divided into two parts, representing temperature fluctuation during day and night. More specifically, data retrieved from 9 am to 9 pm were included in the temperature distribution of day, while data obtained from 9 pm to 9 am were included in the temperature distribution of night. Both day and night temperature data were used to build empirical distributions. Overall, four empirical distributions were built per country.

The maximum specific growth rate (μ_{max}) was estimated individually for transportation, day and night storage as a function of temperature and pH, and a gamma model was applied as follows:

$$\mu_{max} = \mu_{opt} * \gamma(\mathrm{T}) * \gamma(pH) \tag{16}$$

The term γ(T) represents the Cardinal model with Inflection proposed by Rosso et al. (1993) for temperature and it written according to the following equation:

$$\gamma(T) = \frac{(T - T_{min})^2 (T - T_{max})}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)\right]} + \varepsilon_2 \tag{17}$$

$$\varepsilon_2 \ Normal(0, sd_2) \tag{18}$$

Where:

T_{min}, T_{opt} and T_{max} are the theoretical minimum, optimum and maximum values of temperature enabling growth (°C). The cardinal temperature values were retrieved from the study of Kakagianni et al., 2016. The error (ε_2) incorporated in the model was considered as uncertainty due to the limited amount of data.

The γ(T) factor was calculated for transportation, day storage and night storage with T equals to T_{tr}, T_d and T_n, respectively.

The term γ(pH) represents the Cardinal pH model proposed by Rosso et al., 1995 and it was written as follows:

$$\gamma(pH) = \frac{(pH - pH_{min})(pH - pH_{max})}{(pH_{opt} - pH_{min})(pH - pH_{opt}) - (pH_{opt} - pH_{max})(pH_{min} - pH)} + \varepsilon_3 \tag{19}$$

$$\varepsilon_3 \ Normal(0, sd_3) \tag{20}$$

Where:

pH_{min}, pH_{opt} and pH_{max} are the theoretical minimum, optimum and maximum values of pH enabling growth. The cardinal pH values were previously reported (Misiou et al., 2021). The error (ε_3) incorporated in the model was considered as uncertainty due to the limited amount of data.

The optimum specific growth rate (μ_{opt}) of several plant-based milk alternatives obtained on the strain ATCC7953 was reported by Misiou et al., 2021. The variability of the optimum specific growth rate of the products was described through an empirical distribution, a non-parametric bootstrap procedure was carried out to capture the uncertainty.

In order to estimate the quantity of bacteria per pack surviving the UHT treatment and able to grow during distribution and storage (N_f) the total growth was estimated as the sum of growth during transportation and storage at consumer stage (Eq. 21–23).

$$N_{trans} = N_p * \exp(\mu_{max} * t_{trans}) \tag{21}$$

Where:

μ_{max} is derived from Eq. (16) with $\mathrm{T} = \mathrm{T}_{tr}$.

The growth during storage at consumer stage was estimated as an equivalent of 60 days and 60 nights as follows:

$$N_{night} = N_{trans} * \exp(\mu_{max} * t_{night}) \tag{22}$$

Where:

μ_{max} is derived from Eq. (16) with $\mathrm{T} = \mathrm{T}_n$

$$N_f = N_{night} * \exp(\mu_{max} * t_{day}) \tag{23}$$

Where:

μ_{max} is derived from Eq. (16) with $\mathrm{T} = \mathrm{T}_d$.

The final microbial concentration (C_{final}) in each pack (250 ml) was estimated as follows:

$$C_{final} = \frac{N_f}{250} \tag{24}$$

2.2. Model implementation

The spoilage risk assessment model was implemented in R software (R Core Team, 2022). Fitting of distributions was performed by using the fitdistrplus package (Delignette-Muller & Dutang, 2015). The second order Monte Carlo simulation, that was used to propagate uncertainty and variability separately, was carried out using the mc2d package (Pouillot & Delignette-Muller, 2010). The number of iterations performed for uncertainty was 1,000 and for variability 10,000. Results are reported with their confidence interval capturing the uncertainty dimension.

2.3. Sensitivity analysis

A sensitivity analysis was performed in order to assess the impact of probabilistic inputs on the model outcome and the associated risk of spoilage. The sensitivity analysis was performed separately for Greece and Poland. The tornado function of the mc2d package was carried out with the Spearman rank correlation method; the impact of uncertainty was assessed through the confidence interval around the correlation coefficient values.

2.4. Climate change scenario

A climate change scenario (CCs) was designed, based on the projections provided by the Intergovernmental Panel on Climate Change (IPCC, 2021). More specifically, the climate change scenario includes an increase of temperature of 2 °C, which was assumed to be homogeneously distributed in the hourly temperature profiles of the baseline scenario for both countries. The results were expressed as relative risk compared to the baseline scenario (current climatic conditions) in the two countries, respectively.

2.5. Mitigation strategies

Two alternative strategies were designed to mitigate the risk of spoilage of plant-based milk alternatives due to climate change. These strategies evaluated the risk of spoilage considering the impact of the process conditions and the distribution and storage conditions by modifying the inputs of Table 1. A brief description of the mitigation strategies is provided below.

2.5.1. UHT treatment intensity

The effect of UHT treatment duration (3 and 7 sec) and temperature (140 and 145 °C) on the risk of spoilage of a plant-based milk alternative that distributed with P_{truck} equals to 50% and stored in Poland and Greece under the climate change scenario during summertime were evaluated.

2.5.2. Use of insulated trucks for distribution

The effect of the use of insulated trucks (25 °C) for distribution on the risk of spoilage of a plant-based milk alternative was evaluated by considering P_{truck} 50 and 100%. The product was UHT-treated at 140 °C for 3 sec prior distribution in Greece and Poland during summertime under the projected climatic conditions.

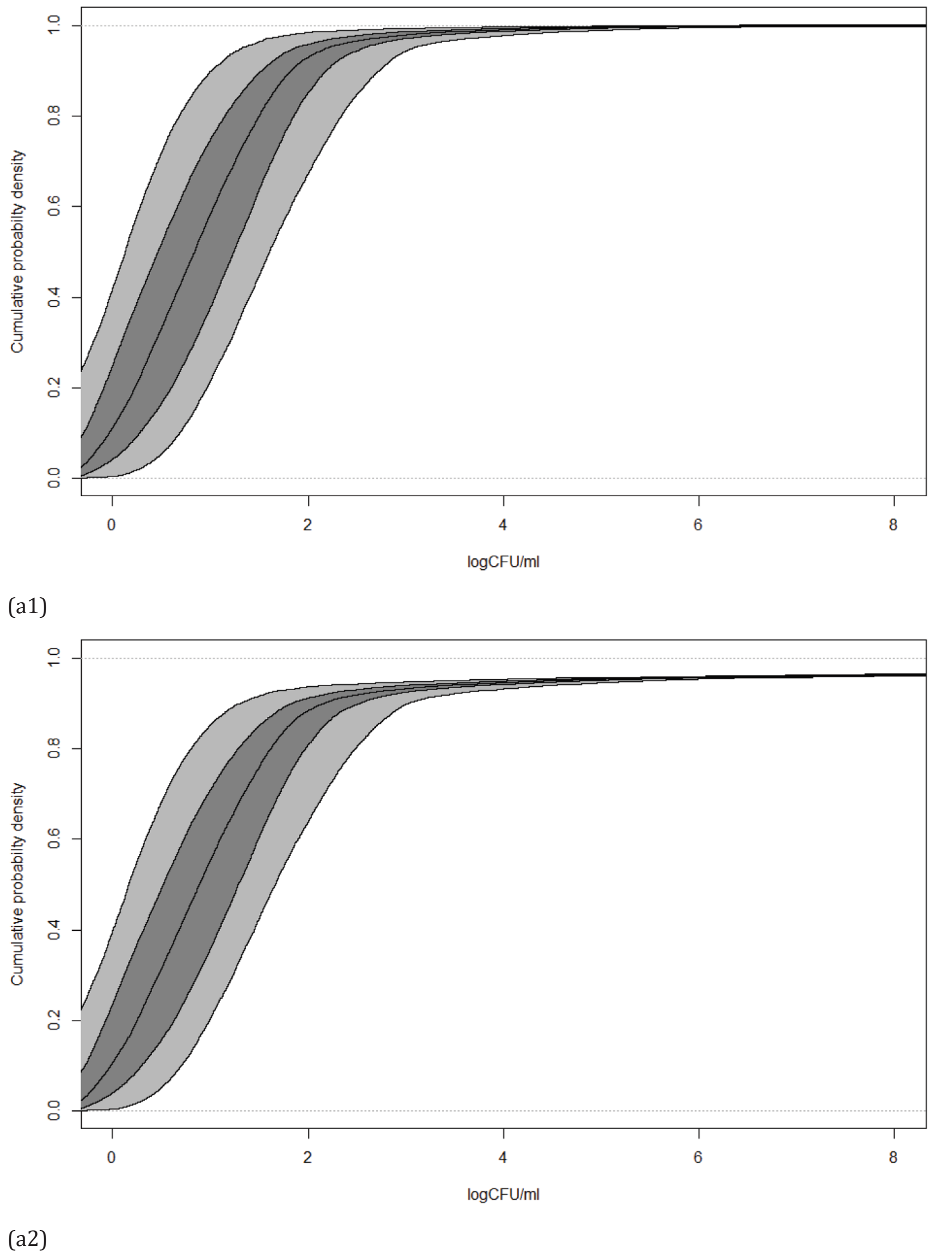


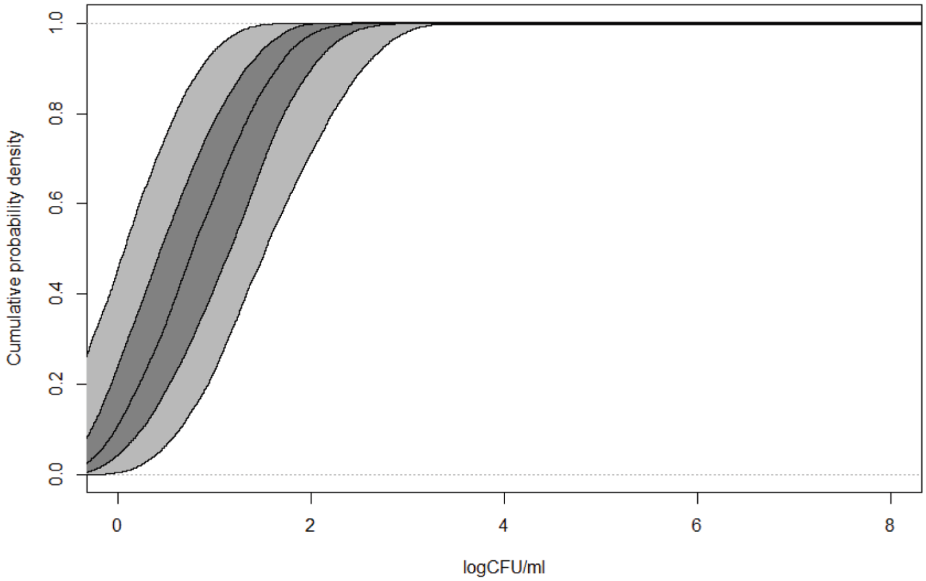
Fig. 2. Cumulative probability distribution of *Geobacillus stearothermophilus* in plant-based milk under the current climatic situation during summertime in **a.** Greece (with (a_1) and without A/C (a_2) at consumer stage) **b.** Poland after 4 months storage at consumer stage. The light grey corresponds to the lower and upper limits of the 95% uncertainty interval, the dark grey corresponds to the 25th and 75th percentiles of the uncertainty.

3. Results

3.1. *Level of Geobacillus stearothermophilus in plant-based in Europe and associated risk of spoilage under the current climatic conditions*

The results of the 2nd order Monte Carlo simulation estimating the final concentration (log CFU/mL) of *G. stearothermophilus* in the plant-based milk alternatives distributed and stored in South and North Europe during summertime under the current climatic conditions are illustrated in Fig. 2**a and 2b**. The predicted final concentration (log CFU/mL) of *G. stearothermophilus* in a plant-based milk alternative distributed and stored in Poland had a mean value of 0.8 with a 95th confidence interval of (0.1;1.5). In Greece, the mean value of the final concentration of the microorganism was 0.9 (0.3; 1.6) with an A/C system in place (Fig. 2**a1**) and 2.5 (1.9; 3.3) without an A/C system (Fig. 2**a2**).

The estimated risk of spoilage due to the growth of *G. stearothermophilus* in plant-based milk alternatives transported and stored in North (Poland) and South Europe (Greece) under the current climatic conditions is presented in Table 2. As expected, there is no risk of spoilage during the wintertime for both countries since the recorded historical temperature for 2022 did not exceed the minimum growth temperature of *G. stearothermophilus*. On the contrary, based on the recorded temperature data growth was observed in both countries during summertime. Interestingly, the final concentration of *G. stearothermophilus* found in a plant-milk alternative distributed and stored in Poland did not exceed the N_{max}, and therefore no risk of spoilage was estimated. In Greece, in contrast, growth was predicted to exceed the N_{max} regardless of the A/C system at the consumer stage. However, the risk of spoilage was lower when the A/C system was



(b)

Fig. 2. (*continued*).

Table 2
Risk of spoilage due to growth of *Geobacillus stearothermophilus* in plant-based milk alternatives distributed and stored in North and South Europe under the current climatic conditions.

Period	Country	Estimate values	2.5 % Confidence Intervals	97.5 % Confidence Intervals
Winter	Poland	–	–	–
	Greece	–	–	–
Summer	Poland	–	–	–
	Greece (without A/C at consumer stage)	6.2×10^{-3}	2.3×10^{-3}	1.1×10^{-2}
	Greece (with A/C at consumer stage)	1.0×10^{-4}	0	4.0×10^{-3}

assumed to be in place (1.0×10^{-4} 95% CI (0; 4.0×10^{-3})), compare to the risk of spoilage when no A/C was assumed at the consumer stage (6.2×10^{-3} 95% CI (2.3×10^{-3};1.1×10^{-2})).

3.2. Influence of model inputs on the estimation of the current risk

First of all, the impact of probabilistic inputs on risk of spoilage was assessed through a sensitivity analysis. The analysis was performed separately for Greece and Poland, although only result for Greece is reported in Fig. 3**a** as there was no risk of spoilage in Poland under current climatic conditions. The prevalence (0.14 95% CI (0.12; 0.16)) and concentration in raw material contamination (0.11 95% CI (0.07; 0.15)) along with the temperature of storage (0.16 95% CI (0.09; 0.22)) play a role on the risk of spoilage due to *G. stearothermophilus* in the plant-based milk alternatives in summertime (Fig. 3**a**). However, these three variables cannot be considered as mitigation strategies options since their control is not straightforward. Therefore, to identify and evaluate potential mitigation strategy options, a second sensitivity analysis was performed.

The impact of probabilistic inputs on the final concentration of *G. stearothermophilus* in plant-based milk alternatives, in case of contamination (prevalence artificially set to 100%) is presented in Fig. 3**b**. The variability related to the heat treatment has the highest impact (0.87 95% CI (0.86; 0.88)) on the final concentration of *G. stearothermophilus* in the plant-based milk alternatives. As illustrated in the tornado plot, the uncertainty dimension is relatively narrow (0.86; 0.88). This small impact of the uncertainty included in the heat treatment can be explained by the large D-value dataset used for the fitting. The storage temperature during day at consumer stage (0.07 95% CI (0.06; 0.08)) and the temperature during transportation (0.15 95% CI (0.14; 0.16)) also played a role. In fact, this latter input revealed an impact of the percentage of insulated trucks, which may be assessed as mitigation strategy option. In contrast, the optimum specific growth rate of the microorganism and the storage temperature during the night had limited impact on the estimated concentration.

3.3. Prediction of spoilage risk under climate change scenario

A climate change scenario (CCs), which includes an increase in temperature of 2 °C, was designed to predict the risk of spoilage under climate change conditions. The predictions were made separately for the two countries, and the results are presented in Table 3. Based on the estimations presented in Table 3, 1 out of 10,000 product units distributed and stored in Poland during summertime may exceed the spoilage level under climate change conditions (zero under current climatic conditions). Concerning the risk of spoilage in Greece, the probability of exceeding the N_{max} is almost twice bigger for the consumers who have an A/C system in place and three times bigger for those who do not possess an A/C system.

3.4. Mitigation strategies

In order to reduce the estimated risk of spoilage of plant-based milk alternatives under climate change conditions, two mitigation strategies were investigated. These strategies evaluated the risk of spoilage considering the most impactful inputs of the risk assessment model based on the sensitivity analysis results. Hence, the impact of the process as well as the percentage of insulated trucks was studied.

The effect of UHT treatment temperature (140 and 145 °C) and duration (3 and 7 sec) on the risk of spoilage of a plant-based milk alternative that distributed and stored in Poland and Greece with 50 % insulated trucks were evaluated. According to Table 4, the application of a UHT treatment at 140 °C for 3 sec failed to significantly reduce the risk

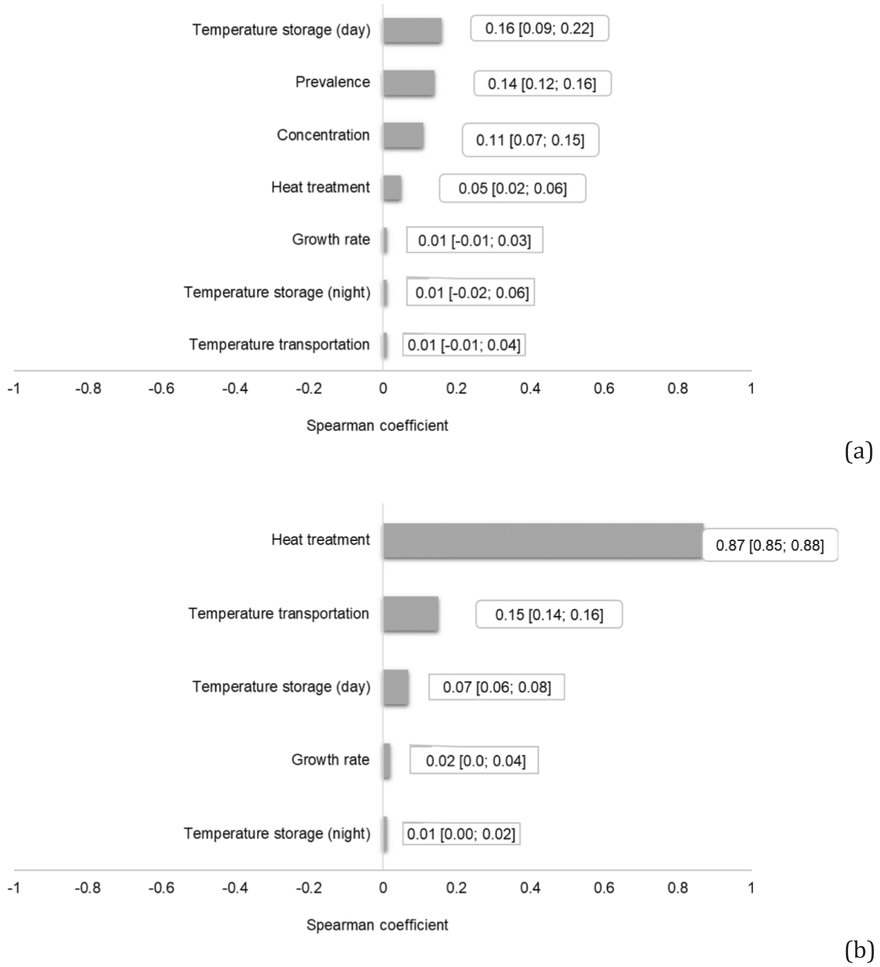


Fig. 3. Tornado plot illustrating the sensitivity analysis of all the variable inputs on the risk of spoilage **(3a)** and on the final concentration (LogN) (**3b**) of *Geobacillus stearothermophilus* in plant-based milk alternatives in Greece during summertime. Spearman coefficient estimates with 95% uncertainty interval in bracket.

Table 3
Risk of spoilage due to growth of *Geobacillus stearothermophilus* in plant-based milk alternatives under climate change scenario during summertime in Greece and Poland. Estimated values and 95% confidence interval.

Country	Risk of spoilage under climate change scenario
Poland[a]	1.0×10^{-4} (0; 5.0×10^{-4})
Greece (without A/C at consumer stage)[b]	3.0 (-;2.75)
Greece (with A/C at consumer stage)[b]	2.0; 2.35)

[a] For Poland, the risk is expressed in absolute value as the risk of spoilage was estimated to zero under current climatic conditions.

[b] For Greece, the risk is expressed as a relative increase in comparison with the current climatic conditions. For instance, 2 means a risk multiplied by 2 in comparison with current climatic conditions.

Table 4
Impact of mitigation strategies on the relative risk of spoilage due to growth of *Geobacillus stearothermophilus* in plant-based milk alternatives under climate change scenario. Estimated values and 95% confidence interval.

Mitigation strategy	Input values	Poland	Greece (without A/C at consumer stage)	Greece (with A/C at consumer stage
UHT treatment intensity	3 sec at 140 °C[a]	1.0 (-;0.6)	1.0 (0.99; 1.03)	0 (-;2.18)
	3 sec at 145 °C[a]	0.5 (-;0.66)	0.58 (0.54; 0.63)	0 (-;1.0)
	7 sec at 140 °C[a]	0 (-; 0.60)	0.78 (0.76; 0.78)	0.33 (-;1.0)
	7 sec at 145 °C[a]	0 (-; 0.20)	0.18 (0.15; 0.20)	0 (-;0.18)
Insulated transportation (P_{truck})	50 %[b]	1.0 (-;0.80)	0.95 (0.90; 0.96)	0. (-;1.18)
	100 %[b]	0 (-;1.0)	0.95 (0.89; 0.92)	–

[a] $P_{truck} = 50\%$ [b] *UHT (3 sec at 140 °C).*

of spoilage. In the same vein, an increase on the temperature intensity (145 °C) for the same duration as the reference conditions (3 sec) could significantly decrease the risk of spoilage in Poland but not in Greece. The spoilage risk was estimated to be only significantly reduced in both countries when the UHT treatment is performed at 145 °C for 7 sec.

The effect of insulated trucks transporting of plant-based milk alternative from the factory to the retailers on the risk of spoilage was evaluated by considering a percentage of insulated trucks equals to 50 and 100%. Products were UHT treated at 140 °C for 3 sec prior to distribution in Greece and Poland during summertime under the designed climate change scenario. As presented in Table 4, an increase in the number of insulated trucks reduce the risk of spoilage in both studied countries.

4. Discussion and conclusions

To the best of our knowledge, this is the first attempt to employ a quantitative microbial spoilage risk assessment (QMSRA) in plant-based milk alternatives (PBMA). Hence, the results of the present study are only comparable to the QMSRA performed to evaluate the risk of spoilage due to the growth of *Geobacillus stearothermophilus* in milk, milk powders and other relevant food products ie. canned beans. The estimated risk of spoilage due to the growth of *G. stearothermophilus* in PBMA is significantly higher compared to the risk of spoilage of UHT-type products reported by Pujol and colleagues (Pujol et al., 2015). The observed difference is mainly attributed to the fact that a significantly lower initial contamination level and a more severe heat treatment was assumed in the latter study. In the same vein, the risk of spoilage estimated for the PBMA is notably higher compared to the risk of canned milk reported by Koutsoumanis et al. (2022). However, the risk of spoilage of PBMA is relatively lower compared to the non-sterility incidences in canned green beans which was reported to reach 0.5 % (Rigaux et al., 2014).

The risk of spoilage due to the growth of *G. stearothermophilus* in PBMA transported and stored in North (Poland) and South Europe (Greece) under the current climatic conditions was estimated as the baseline scenario in this study. As expected, there is no risk of spoilage during wintertime for both countries since the recorded historical temperature data for 2022 did not exceed the minimum temperature of growth of *G. stearothermophilus*. On the contrary, current temperature conditions during summertime allowed growth in both countries. Yet, the total growth of the spoiler in a PBMA distributed in Polish market did not exceed the spoilage level ($10^{7.5}$ CFU/mL) and therefore the risk of spoilage is considered negligible. Our results are in line with the results of Kakagianni and Koutsoumanis in which the marginal ability of the current temperature conditions in controlling spoilage of evaporated milk due to the growth of *G. stearothermophilus* in Mediterranean was highlighted (Kakagianni & Koutsoumanis, 2018).

The upcoming increase in global mean surface temperature due to climate change is expected to increase the risk of spoilage of products that are distributed and stored at ambient temperature conditions. Based on the evidence provided in the present study, a temperature increase by 2 °C will almost double the risk of spoilage of plant-based milk alternatives distributed and stored in South Europe, especially when there is no A/C system in place at consumer level. The results presented in the study confirm the potential increase of spoilage incidence due to climate change in hot climate regions and temperate climates (Kakagianni and Koutsoumanis, 2018; Misiou et al., 2021; Misiou and Koutsoumanis, 2021; Koutsoumanis et al., 2022).

Against this background, controlling the risk of spoilage of PBMA deem to be crucial and therefore, several mitigation strategies should be investigated. In the present study, two mitigation strategies were investigated under climate change conditions, namely the increase of the heat treatment intensity and the use of insulated trucks for the distribution of the products. Based on the results, both the increase of the heat treatment intensity and the use of insulated trucks can lead to a significant reduction of the risk. Taking all the above into consideration, the food business operators might be forced either to modify their production lines or to ensure the prevention of growth during distribution though a major change in their logistics, especially when they aim to place their products in the southern European market during summertime.

This study should be seen in light of its limitations, since the uncertainties associated with the assumptions made along with the data used in this model could affect the estimation of the risk. Most of uncertainties have been quantified and introduced into the model, alongside the variability, through the use of 2nd order Monte Carlo simulation. However, some uncertainties could not be quantified, but should not be neglected. The latter point is of a great importance especially when the goal of the QMRA is to estimate the absolute risk (EFSA, 2014). For the heat-inactivation module, it was assumed that there was no spore clump, which is likely to be realistic when the pre-process contamination is not too high, which is the case in our study. A second source of non-quantitative uncertainty is the *meta*-regression analysis performed to model heat inactivation. More specifically, the 566 D-values extracted from the literature underwent a $\log_{10}$-transformation prior plotting against temperature. A linear regression model was fitted into the $\log_{10}$-transformed D-values by assuming a log linear pattern. The uncertainty included in the risk model only considered the fitting error while the error due to inactivation curve was neglected (error in data generation and/or error in primary model fitting) (Haas et al., 2014). An additional source of non-quantitative uncertainty is the use of outdoor temperature data to approximate the indoor temperature at consumer stage. Through this approximation, the indoor temperature was slightly overestimated and subsequently led to an overestimation of the risk estimates. Nevertheless, the present study was focused on the increasing risk of spoilage under climate change scenario and therefore estimating the absolute risk was not the purpose.

The present study was further limited by the fact that the probability of outgrowth was not considered. More specifically, it was assumed that all surviving spores were able to germinate and outgrowth after the heat treatment. This assumption was made due to the lack of data on the probability of outgrowth of *G. stearothermophilus* in the plant-based milk alternatives. The risk of spoilage reported here may be then overestimated, reason why it is more relevant to interpret the result in relative term than in absolute values. Nevertheless, the extension of the model through the incorporation of additional parameters related to outgrowth may increase the uncertainty. Hence, the estimated risk of spoilage presented in this study might be re-assessed in the presence of the above-mentioned data.

In conclusion, the quantitative microbial spoilage risk assessment (QMRSA) of plant-based milk alternatives by *G. stearothermophilus* developed in this study can form the basis for a risk management of these products by quantify the potential risk under current climatic conditions and climate change scenarios while at the same time providing promising mitigation strategies for the food business operators.

CRediT authorship contribution statement

Ourania Misiou: Conceptualization, Data curation, Software, Writing – original draft, Visualization. **Konstantinos Koutsoumanis:** Conceptualization, Methodology, Software, Supervision, Writing – review & editing. **Jeanne-Marie Membré:** Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112638.

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