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PROTECT

Quantitative Microbial Spoilage Risk Assessment: Sensitivity and Scenario analysis

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Abstract

Globally, studies of climate impacts, mitigation, and adaptation strategies are increasingly becoming major areas of scientific interest. Such studies are the foundation of evidence-based policy making. Scientific models serve as the bridge between science and policy. As all models are approximating reality, quantifying the uncertainties involved is a significant step that needs to be implemented if models are to benefit society. Scenario and sensitivity analysis are methodologies that tackle the issue of quantifying uncertainty. This document highlights the importance of sensitivity and scenario analysis as demonstrated in two applications. The first application demonstrates the necessity of performing scenario and sensitivity analysis by using a probabilistic approach to address uncertainty concerning the input parameters of a Quantitative Microbial Spoilage Risk Assessment (QMSRA) in two case studies. The second part deals with the climate change scenarios proposed by the Intergovernmental Panel on Climate Change (IPCC). More specifically it can be considered a tutorial on preparing suitable climate change scenarios to assess food safety locally. Furthermore, it underlines the importance of partitioning the uncertainty in its different sources.

Introduction

One of the most significant issues of the 21st century is to provide sufficient food for the growing population while sustaining the already stressed environment, which is endangered by climate change. Globally, studies of climate impacts, mitigation, and adaptation strategies are increasingly becoming major areas of scientific interest, e.g. impacts on food quality, animal health, production of crops such as corn and wheat, and water resources (Kang et al., 2009). Such studies are the foundation of evidence-based policy making.

When the future is inherently uncertain, decision-makers can recognize change more quickly and effectively using scenario analysis instead of a detailed projection of the most likely future. In general, integrated models of complex socio-ecological systems are increasingly used to assess the future of food industries under key factors such as future climates, population dynamics, as well as demand and supply for food and energy (Gao et al., 2016). Most importantly, scientific models serve as the bridge between science and policy. As all models are approximating reality, quantifying the uncertainties involved is a significant step that needs to be implemented if models are to benefit society.

Typically, uncertainty can be classified into two categories: aleatory or epistemic. Aleatory uncertainty or variability is uncertainty that exists because the system and/or the environment under study have inherent variations. When it comes to food microbiology, aleatory uncertainty is known as biological variability. If sufficient information is available, this kind of uncertainty can be quantified but never reduced. Then, the relevant variables are defined through probability distributions. As a result, probabilistic modelling approaches are frequently utilized to address aleatory uncertainty. This kind of uncertainty is also referred to as intrinsic, irreducible, and stochastic. On the other hand, epistemic uncertainty results from the lack of knowledge. This uncertainty does not always lead to errors. For example, a model may be accurate for some, less complex, systems, even though it does not describe all the dynamics of the system. By gaining more insight into the system dynamics, epistemic uncertainty can be reduced. Thus, reducible, subjective, and cognitive uncertainty are terms used to describe epistemic uncertainty (Nimmegeers, 2018).

Scenario and sensitivity analysis are methodologies that tackle the issue of quantifying uncertainty. In particular, scenario analysis aims to assess the uncertainty of model predictions originating from uncertainty in model inputs without attempting to characterize the different sources of uncertainty. In contrast, sensitivity analysis focuses on determining the relative contributions of multiple inputs and/or parameters to the uncertainty of the output (Saltelli et al., 2010). In this regard, sensitivity analysis should not be viewed as a substitute for scenario analysis but as an enhancement of it.

This document highlights the importance of sensitivity and scenario analysis as demonstrated in two applications. The first application demonstrates the necessity of performing scenario and sensitivity analysis by using a probabilistic approach to address uncertainty concerning the input parameters of a Quantitative Microbial Spoilage Risk Assessment (QMSRA) in two case studies. The first case study uses a probabilistic model to perform scenario analysis in the context of climate change. The second case study utilized another QMSRA probabilistic model covering the food chain to conduct sensitivity analyses. The second part deals with the climate change scenarios proposed by the Intergovernmental Panel on Climate Change (IPCC). More specifically it can be considered a tutorial on preparing suitable climate change scenarios to assess food safety locally. Furthermore, it underlines the importance of partitioning the uncertainty in its different sources.

Scenario and Sensitivity Analysis for QMSRA

The sensitivity analysis in this document determines which input parameters affect the output of the QMSRA model the most. Based on the sensitivity analysis results, alternative scenarios can be implemented by modifying the most impactful parameters of the model, and mitigation strategies can be investigated to reduce the associated risk. Sensitivity and scenario analyses are employed in two case studies related to *Geobacillus stearothermophilus* growth in canned milk and plant-based milk alternatives, respectively.

Case study A: Geobacillus stearothermophilus in canned milk

Background

Microbiologically stable or shelf-stable foods include products which will not spoil or cause disease when stored at ambient temperature or “on the shelf”. They represent a major group in the food market and include products of high consumption such as canned foods, products processed with Ultra High Temperatures (i.e. UHT milk), pasteurized acidic foods (fruit and vegetable juices), dried foods etc. Shelf-stable foods are not sterile, but the microorganisms present cannot grow in the food environment during distribution and storage to levels that can cause spoilage. For example, canned foods undergo a severe heat process, which is primarily designed to destroy spores of pathogenic Clostridium botulinum (Jay et al., 2008; Membré and van Zundijlen, 2011). Other spoilage spore-forming bacteria, however, are more heat resistant and can survive the thermal process designed to control this pathogen. Despite this microbial contamination, these foods are considered shelf-stable because the survivors of the heat process are thermophilic and require a certain storage time at high temperatures in order to grow to spoilage levels (André et al., 2013; Bevilacqua et al., 2009; Gocmen et al., 2005; Rigaux et al., 2014). Such time-high temperature conditions are currently very rare under normal distribution and storage conditions prevailing in regions with temperate climates (Kakagianni and Koutsoumanis, 2018; Misiou and Koutsoumanis, 2021). Considering the climate change and the expected increase of the planet's temperature (IPPC, 2021; Morice et al., 2012; Räisänen et al., 2004; UNFCCC, 2014), however, the question arises is how marginal the latter limiting condition for spoilage is and whether global warming can threaten the microbiological stability of non-refrigerated foods. A positive answer to the latter question could lead to significant social-economic consequences, requiring a high level of preparedness by both the food industry and policymakers.

The IPCC projections give likely ranges of global temperature increase in five scenarios for population, economic growth and carbon use (IPPC, 2021). Based on the latter report, the increase of global mean surface temperature by the end of the 21st century (2081 - 2100), relative to 1986-2005, was projected for five different Representative Concentration Pathways (RCP) considering the possible levels of greenhouse gas emissions (GHG). Under the scenario RCP1.9 and 2.6 (very low and low GHG), the global mean surface temperature increase is likely to be 1.0 to 1.8 °C and1.3 to 2.4 °C, respectively. The intermediate GHG scenario RCP4.5, projected a likely increase between 2.1 and 3.5 °C, while the RCP7.0 and RCP8.5, which represent high and very high GHG emissions, projected an increase of 2.8 to 4.6 °C and 3.3 to 5.7 °C, respectively. Raftery and colleagues developed a statistically based probabilistic forecast of CO_2 emissions and temperature change to 2100 and reported that the likely range of global temperature increase is 2.0 - 4.9 °C, with a median 3.2 °C and only 5% and 1% chances that it will be less than 2 °C and 1.5 °C, respectively (Raftery et al., 2017). Considering the regional heterogeneity of temperature increase and the seasonality of warming (Chakraborty et al., 2011; ECDC, 2012; Schmidhuber and Tubiello, 2007; Watkiss, 2013) in any of the above scenarios, the increase in global mean surface temperature is expected to have a significant impact on the temperature conditions in which the shelf-stable foods are exposed during distribution and storage with a potential effect in their microbiological stability.

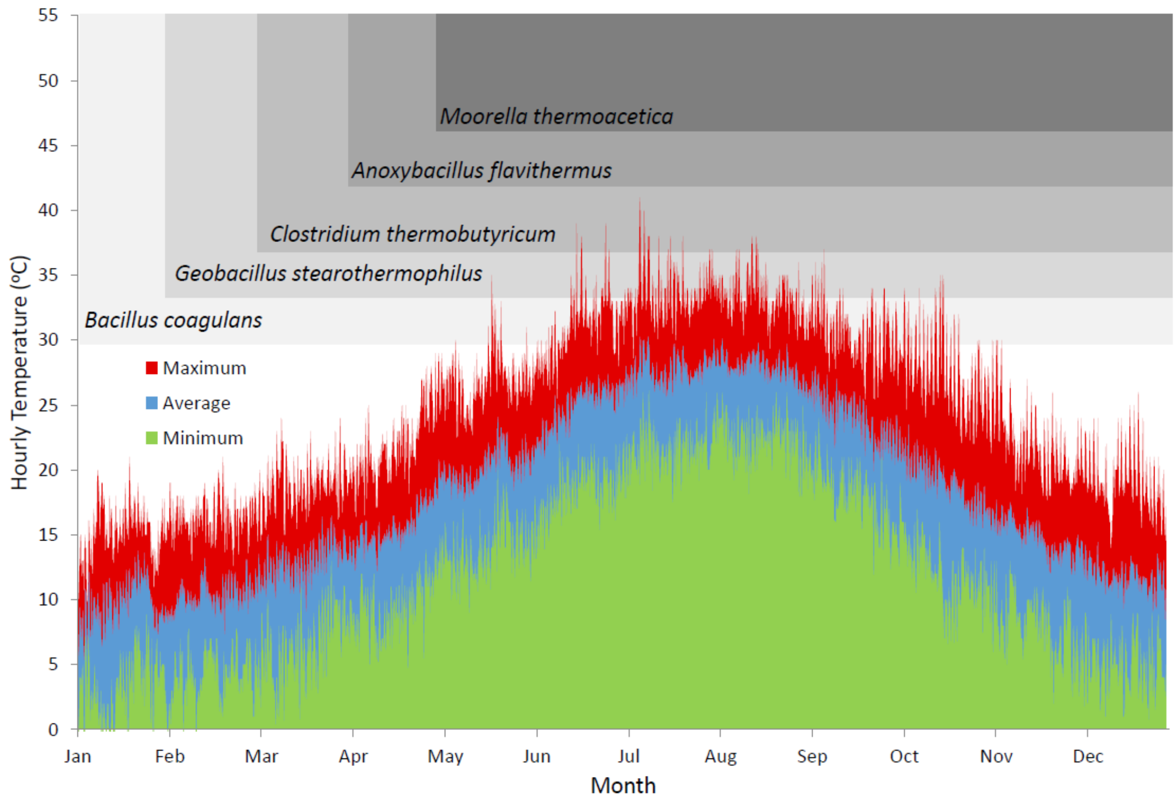


Figure A1. Ambient temperature in Athens, Greece, during the period 2011-2020. Grey areas show the temperature region allowing the growth of the most important thermophilic spore-forming spoilage bacteria of non-refrigerated foods.

To address our hypothesis, we compared annual ambient temperature profiles from 38 European cities for 10 recent consecutive years (2011 - 2020) with the minimum temperatures for growth of thermophilic bacteria commonly present in shelf-stable foods and showed that the current temperature conditions are approaching their growth range for the Southern European region. **Figure A1** presents an example for Athens (Greece), where the temperature conditions during the last 10 years is marginally lower compared to the minimum temperature for growth of the most important spoilers of non-refrigerated foods including *Bacillus coagulans, Geobacillus stearothermophilus, Clostridium thermobutyricum, Anoxybacillus flavithermus and Moorella thermoacetica* (André et al., 2013; Byrer et al., 2000; De Clerck et al., 2004; Kakagianni et al., 2016; Wiegel et al., 1981). These results indicate that a further temperature increase, due to global warming, may allow bacterial growth at levels that can cause spoilage. Among the thermophilic spore-forming bacteria, *G. stearothermophilus* constitutes the most important quality problem for the food industry in relation to thermally processed shelf-stable heat processed food products (André et al., 2013; Carlier and Bedora-Faure, 2006; Carlier et al., 2006). The high prevalence and concentration of spores in the raw materials, the adhesive characteristics of spores that enhance their persistence in industrial environments and, most importantly, the extreme heat-resistance of its spores are among the major factors explaining the importance of this thermophilic endospore-former (André et al., 2013; Postollec et al., 2012; Yoo et al., 2006). Hence, in the present work, we evaluate the potential effect of climate change on the microbiological stability of canned milk as a shelf-stable food case study by using a quantitative risk assessment model. The model uses predictive microbiology tools to assess the effect of 3 global warming projections (temperature increase of 1.5, 3.0 and 4.5 °C) on *G. stearothermophilus* growth and further translate it to the risk of canned milk spoilage. In addition, we evaluate the impact of insulated non-refrigerated distribution and storage as a strategy to mitigate the increased risk of spoilage due to climate change.

Materials and Methods

Temperature data

Historical hourly temperature data covering 38 European cities for ten consecutive years over the period 2011-2020 was obtained from the Weather Underground database (http://www.wunderground.com/). Data were collected from airport weather stations of European capital cities. Slovenia, Spain, Cyprus and Israel have no stations in their capital city; therefore, temperature data was collected from the next highly populated city. The temperature data were recorded at 20 and 30 min intervals, depending on the station. In total, about 10 million temperature records corresponding to 380 annual temperature profiles were obtained and used in the study.

Predicting Geobacillus stearothermophilus growth in canned milk under global warming scenarios

The predictive model of Kakagianni and colleagues (Kakagianni et al., 2016) describing the effect of temperature on the growth of *G. stearothermophilus* was applied to assess the spoilage risk of canned milk during distribution and storage in the market of 38 European capitals. The growth prediction was based on the combination of the secondary Cardinal Model with Inflection (Rosso et al., 1993) with the differential equations of the Baranyi and Roberts primary model (Baranyi and Roberts, 1994). The effect of temperature on the maximum specific growth rate (μ_{max}) was predicted by the secondary model as follows:

$$\mu_{\max}(T) = 2.068 \cdot \rho(T) \qquad \text{(Eq. 1)}$$

$$\rho(T) = \begin{cases} 0 & , \quad T \leq 33.76 \\ \frac{(T-68.14)(T-33.76)^2}{(61.82-33.76)([(61.82-33.76)(T-61.82)-(61.82-68.14)(61.82+33.76-2T)]} & , \quad 33.76 \leq T \leq 68.14 \\ 0 & , \quad T \geq 68.14 \end{cases}$$

(Eq. 2)

The prediction of growth under dynamic storage temperature was based on the time-temperature profiles of the European cities, T(t), in conjunction with the secondary model (Eq. (1)) for the estimation of the "momentary" μ_{max} and the differential equations of Baranyi and Roberts model (Eqs. (3) and (4)), which were numerically integrated with respect to time:

$$\frac{d}{dt}x = \mu_{\max\,(T)}\frac{q}{1+q}\left(1-\frac{x}{x_{max}}\right)x \qquad \text{(Eq. 3)}$$

$$\frac{d}{dt}q = \mu_{\max\,(T)}q \qquad \text{(Eq. 4)}$$

The parameter q denotes the concentration of a substance critical to the growth and is related to the physiological parameter a_0, as follows:

$$a_0 = \frac{q_0}{1+q_0} \qquad \text{(Eq. 5)}$$

The model was based on the assumption that the growth rate is adopted instantaneously to the new temperature environment after a temperature shift. For the baseline scenario (BSc), hourly temperature data from 38 European cities over a period of ten years (2011-2020) were used as model input data to predict the total growth of *G. stearothermophilus* in canned milk distribution and storage for one year, which is the normal expiration date period of the product. The total growth of *G. stearothermophilus* in canned milk was also predicted for 3 global warming scenarios (GWS), including temperature increases of 1.5 (GWSc-1), 3.0 (GWSc-2) and 4.5 (GWSc-3) °C. The temperature increase in all scenarios was assumed to be homogeneously distributed in the hourly temperature profiles of the baseline scenario for each year. For the growth prediction, the initial and maximum population density was fixed to $10^{-2.4}$ (i.e. 1 cell in 250 mL) and $10^{7.5}$ CFU/mL (Kakagianni et al., 2016). The a_0 was set to 1, representing a scenario with the spoilage organism growing with no lag phase.

Assessing the risk of canned milk spoilage

A probabilistic model which takes into account the variability of the initial contamination level and the spoilage level was developed (Koutsoumanis et al., 2021). The model provides a quantitative estimate of the probability that for a certain product, G. stearothermophilus concentration exceeds the spoilage level and causes spoilage at the end of the shelf life. The initial contamination level of *G. stearothermophilus* (N_0) in canned milk was described using a Pert distribution Pert (Min, Mode, Max) with mode, minimum and maximum values of 1; 0.004 (1 cell in 250 mL); 1000 CFU/mL, respectively, based on historical data from Greek dairy industry. A Pert distribution was also used to describe the total growth (tG) of the spoiler with mode, minimum and maximum values estimated for each city and global warming scenario from the growth model for the ten tested years. In the latter case, the mode was estimated as follows (Vose, 2000):

$$mode = \frac{6*Mean-Min-Max}{4} \quad \text{(Eq. 6)}$$

The spoilage level (N_s) was described by a Normal distribution with a mean value of $10^{7.4}$ CFU/mL and a standard deviation of $10^{0.15}$ CFU/mL (Kakagianni et al., 2016). The risk of spoilage was calculated as the probability that the concentration of the spoiler at the end of one-year shelf life N_{end} ($N_{end}=N_0*tG$) exceeds the spoilage level Ns.

$$Risk\ of\ Spoilage = P(N_0 * tG > N_s) \quad \text{(Eq. 7)}$$

The risk of spoilage was assessed using Monte Carlo simulation with 50.000 iterations with @Risk software (@Risk, Palisade, USA).

Evaluating the impact of insulated distribution and storage on the risk of canned milk spoilage

In order to compare and evaluate the impact of insulated distribution and storage on the risk of canned milk spoilage, outdoor and indoor temperature data were collected for non-insulated and insulated storage rooms and truck containers. Outdoor and indoor temperature was monitored simultaneously for a period of 15 days during the summer period in Greece using electronic data loggers (Cox Tracer, Cox Technologies, Belmont, NC, USA). For storage, a Greek dairy industry recorded the temperature in open shed storage areas (non-insulated) and concrete storage rooms (insulated). For distribution, the temperature was recorded in non-insulated and insulated trucks (with overall heat transfer coefficient

U= 0.4 W/m^2 K) used for food transportation.

The collected outdoor and indoor temperature data were fitted to the following exponential smoothing model in order to estimate the smoothing factor parameter using Excel solver:

$$T_{\mathrm{ind_t}} = (1 - a) * T_{\mathrm{out_t}} + a * T_{\mathrm{ind_{t-1}}} \quad \text{(Eq. 8)}$$

Where $T_{\mathrm{ind_t}}$ is the indoor temperature at time t, $T_{\mathrm{out_t}}$ is the outdoor temperature at time t, $T_{\mathrm{ind_{t-1}}}$ is the indoor temperature at time t-1, and a is the smoothing factor. The smoothing factor parameter for the tested insulated storage rooms and transportation trucks was estimated using Excel solver and ranged from 0.941 to 0.952. The lowest value of the estimated smoothing factor parameters for the storage rooms and trucks, representing the lower level of insulation was further used to transform the historical hourly ambient temperature data covering 38 European cities for ten consecutive years over the period 2011-2020 to insulated storage and distribution temperature using (Eq. (8)). The risk assessment model was rerun based on the transformed temperatures to simulate the impact of insulated distribution and storage on the risk of canned milk spoilage.

Results and Discussion

We assessed the effect of different global warming scenarios to the microbiological stability of shelf-stable foods through a case study of canned milk spoilage by *G. stearothermophilus* in the European region. The spores of the later spoiler can survive the canning process and when are exposed to favourable temperatures (> T_{min} ≈ 33 °C) during distribution and storage they transit from an inactive form to active cells after an irreversible cascade of steps (Kakagianni et al., 2016). The growth of metabolic active cells to critical levels (> 10^7 CFU/mL) results in acidification and further coagulation of canned milk leading to significant economic losses for the dairy industry (Burgess et al., 2010; Kakagianni et al., 2016; Rigaux et al., 2014). The growth of *G. stearothermophilus* in canned milk during distribution and storage in 38 European cities was assessed for a period of one year, which is the normal expiration date period of the product. For the baseline scenario (BSc), growth assessment was based on the combination of a dynamic growth model developed by Kakagianni and colleagues (Kakagianni et al., 2016) with hourly ambient temperature profiles of each city for the last 10 consecutive years (2011-2020). The model was further rerun for 3 global warming scenarios (GWS) including increases in the mean surface temperature of 1.5 (GWSc-1), 3.0 (GWSc-2) and 4.5 (GWSc-3) °C and the results were compared with the baseline scenario (**Figure A2**).

The cumulative probability of *G. stearothermophilus* growth in canned milk during one year of storage in all tested European cities and years for the baseline and the 3 global warming scenarios is presented in **Figure A3**. For BSc, growth is limited with a mean value of 0.2 Log CFU/mL and a 95th percentile of 0.8 Log CFU/mL, confirming that current temperature conditions during distribution and storage in Europe do not allow extensive growth of thermophilic bacteria to levels that can cause spoilage of shelf-stable foods. For the global warming scenarios, the average microbial growth increases to 0.5, 1.2 and 2.3 Log CFU/mL and the 95th percentile to 2.2, 6.0 and 9.8 Log CFU/mL for GWSc-1, GWSc-2 and GWSc-3, respectively.

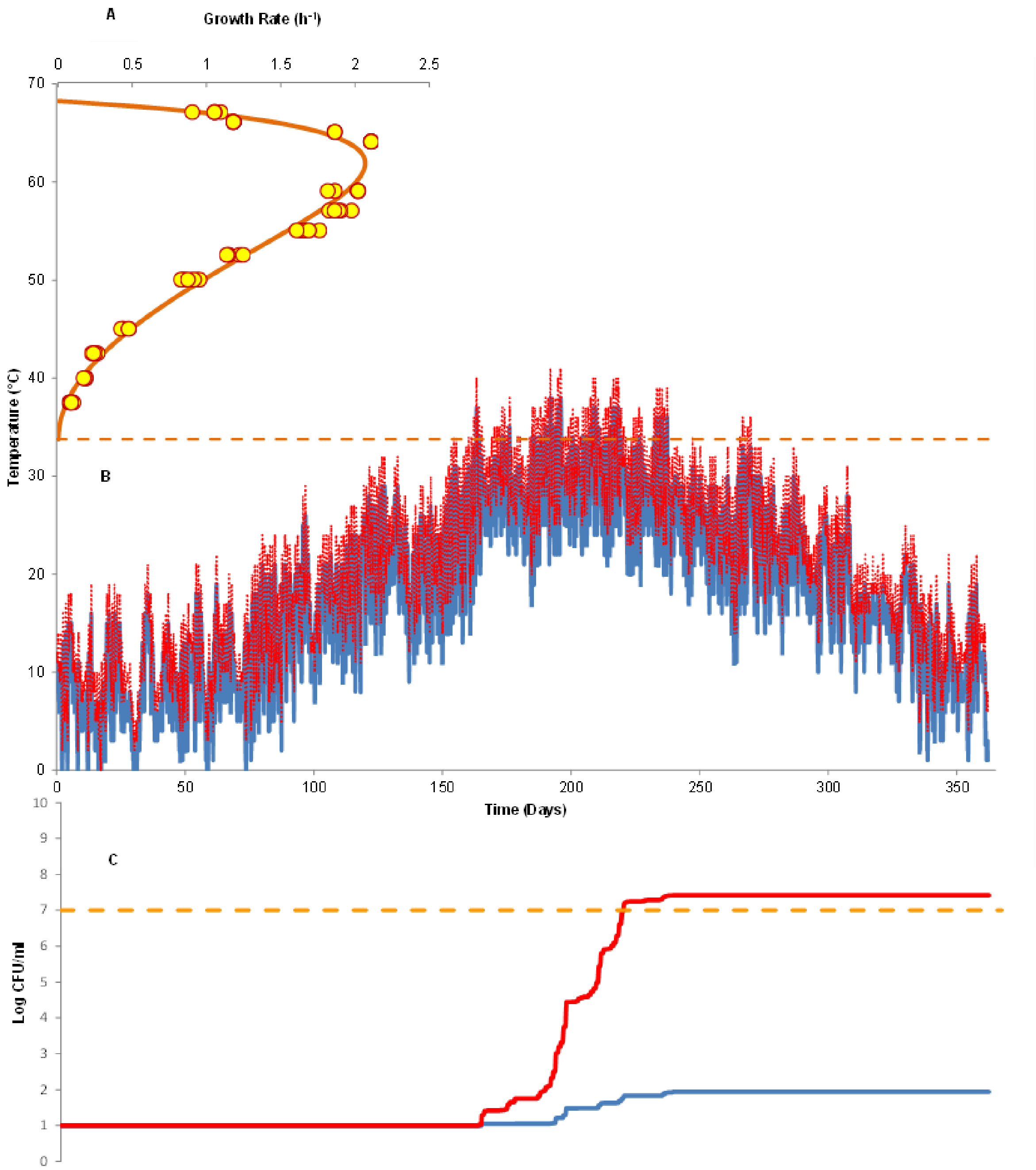


Figure A2. Model development for prediction of *Geobacillus stearothermophilus* growth. **A**: Effect of temperature on the growth rate of *G. stearothermophilus* (Kakagianni and Koutsoumanis, 2018). Points represents observed values and line represents model's prediction. Dotted line indicates the minimum temperature allowing growth of the spoiler. **B**: Ambient temperature recorded in Athens for the year 2015 used for the baseline scenario (blue line). Temperature for global warming scenario 2 (red line) **C**: Predicted growth of *G. stearothermophilus* in canned milk for the baseline (blue line) and the global warming scenario 2 (red line). Dotted line indicates the level of G. stearothermophilus where spoilage is observed.

Microbial growth varies significantly among the tested European cities depending on their temperature conditions (**Figure A4**). For the southern European region, a small temperature increase results to extensive microbial growth to levels that can cause spoilage. The above results confirm the marginal ability of the current temperature conditions in controlling the growth of thermophilic spoilage bacteria in shelf-stable foods while demonstrating that the reported vulnerability of Southern Europe to future climatic changes in relation to food safety and security (ECDC, 2012; Giorgi and Lionello, 2008; Watkiss et al., 2013) is also valid for food spoilage.

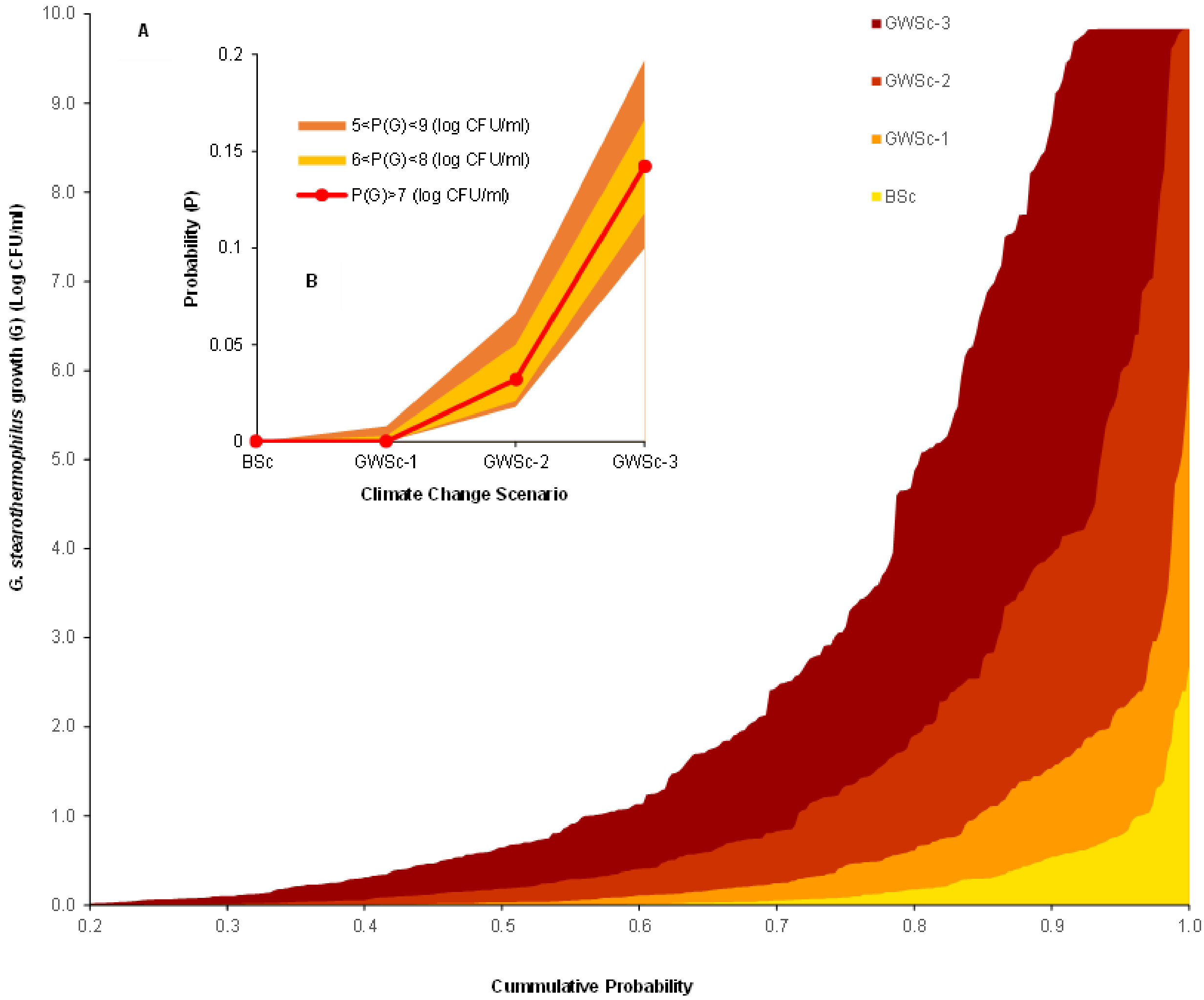


Figure A3. Probability of Geobacillus stearothermophilus growth in canned milk during one year of storage in 38 European cities. A: Cumulative probability of G. stearothermophilus growth for the baseline scenario (BSc) and the 3 global warming scenarios (GWS) including increases in the mean surface temperature of 1.5 (GWSc-1), 3.0 (GWSc-2) and 4.5 (GWSc-3) °C. B: Probability of G. stearothermophilus growth exceeding the spoilage level (7 Log CFU/mL) with 1 and 2 Log CFU/mL intervals.

The data on microbial growth were further translated to risk of spoilage using a probabilistic model, which takes into account the variability on the initial contamination level and the spoilage level (Koutsoumanis et al., 2021). This approach can provide a quantitative estimate of the probability that for a certain canned milk unit *G. stearothermophilus* concentration exceeds the spoilage level at the end of the shelf-life leading to sensory rejection of the product. **Figure A5** shows the simulation results on the concentration of the spoiler at the end of shelf life for the 38 European cities for the BSc and the 3 GWSc. For the BSc the average value of the median and 95th percentiles for all tested cities are 0.2 and 2.1 Log CFU/mL reflecting mainly the variability in the initial contamination level since as shown before (**Figure A3**) the growth for this scenario is very limited. For Northern European cities, global warming projections do not have a significant impact on microbial growth. This can be attributed to the fact that temperature in this region is low and even with an increase of 4.5 °C (GWSc-3) it will not exceed the minimum temperature of growth of *G. stearothermophilus* (T_{min} ≈33.0 °C). In contrast, for the Southern region, global warming may lead to extensive microbial growth. In the case of Rome for example, with a temperature increase by only 1.5 °C (GWSc-1), G. stearothermophilus concentration at end of the shelf life may reach 6.9 Log CFU/mL (99th percentile), approaching the spoilage level of canned milk. For the higher temperature increase projections tested, namely 3 (GWSc-2) and 4.5 °C (GWSc-3), the 99th percentile of *G. stearothermophilus* concentration at the end of the shelf life exceeds the spoilage level in 6and 13 Southern European cities, respectively.

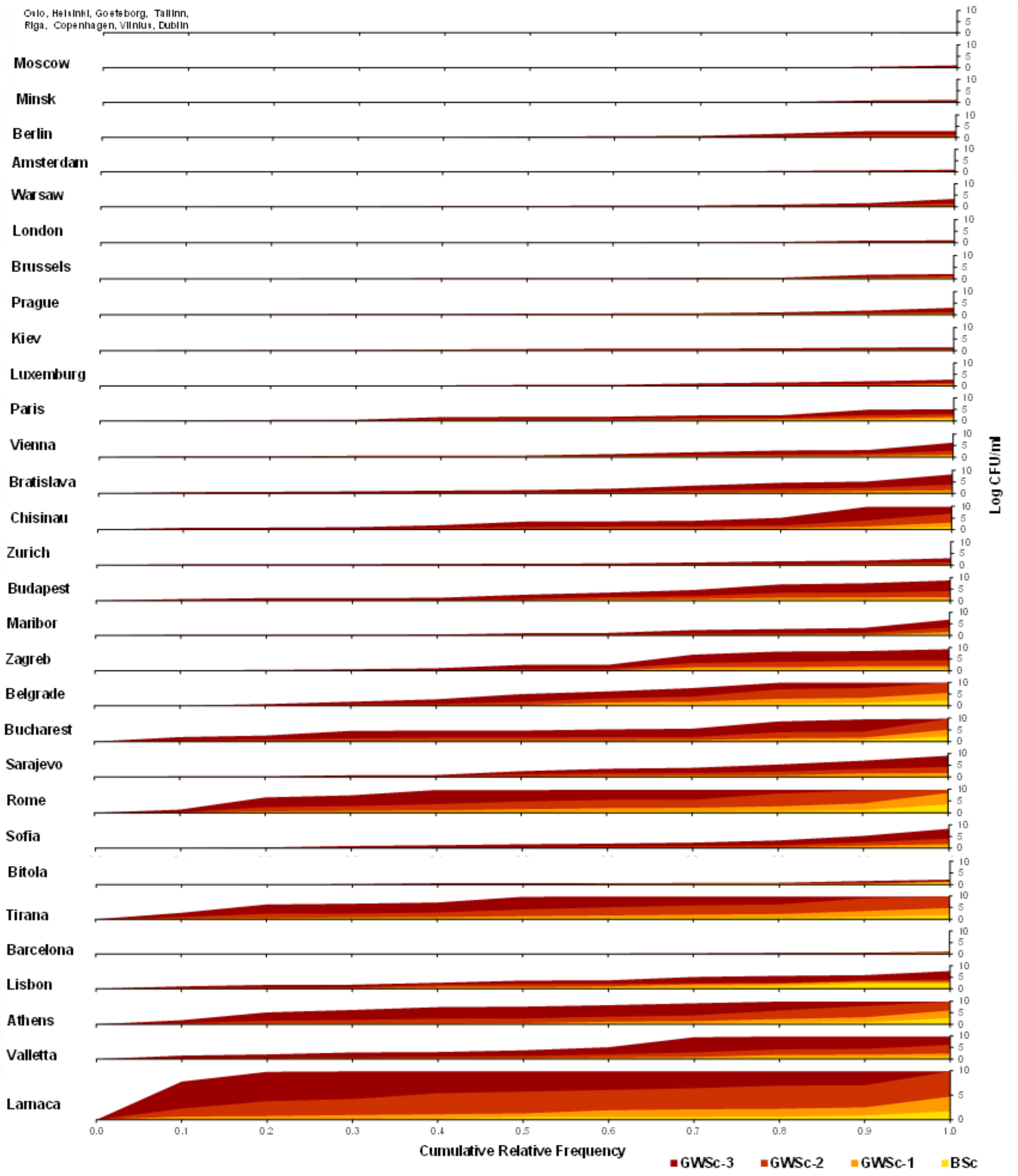


Figure A4. Cumulative probability of *Geobacillus stearothermophilus* growth in canned milk during one year of storage in European cities. Cumulative probability of *G. stearothermophilus* growth for the baseline scenario (BSc) and the 3 global warming scenarios (GWS) including increases in the mean surface temperature of 1.5 (GWSc-1), 3.0 (GWSc-2) and 4.5 (GWSc-3) °C.

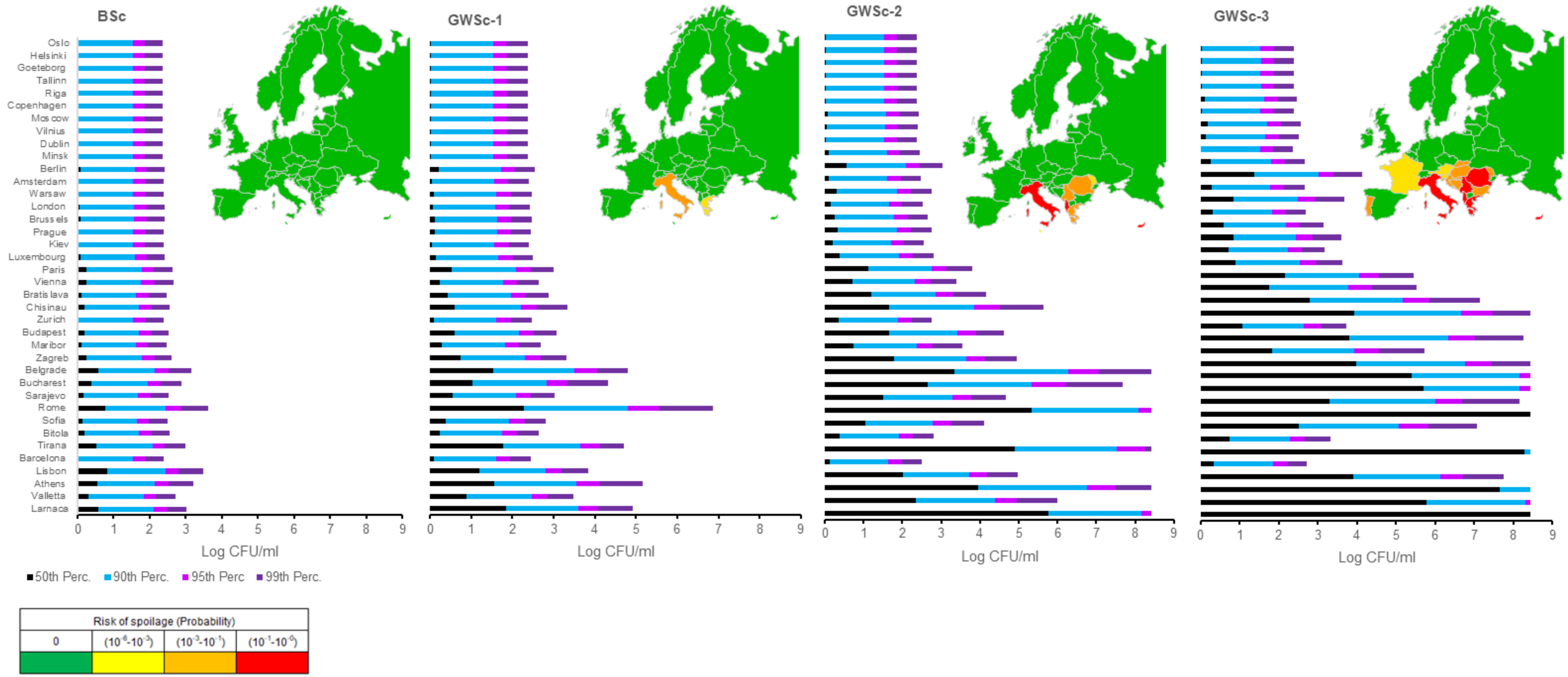


Figure A5. Simulation results on the concentration of *Geobacillus stearothermophilus* and on the spoilage risk assessment. Predicted concentration of *G. stearothermophilus* at the end of canned milk shelf life in 38 European cities for the baseline scenario (BSc) and the 3 global warming scenarios (GWS) including increases in the mean surface temperature of 1.5 (GWSc-1), 3.0 (GWSc-2) and 4.5 (GWSc-3) °C. Maps translate the concentration of *G. stearothermophilus* at end of the shelf life to risk of spoilage as a probability of exceeding the spoilage level.

The risk maps presented in **Figure A5** translate the concentration of *G. stearothermophilus* at the end of the shelf-life to risk of spoilage as a probability of exceeding the spoilage level. For BSc, the risk of spoilage is negligible for all 38 European cities indicating that the temperature conditions recorded during the period 2011-2020 control the growth of the spoiler. For GWSc-1, non-zero risk cities include only Rome and Athens with probability of spoilage 5.3 x 10-3 and 1.6 x 10-5, respectively. The simulation results show that for a temperature increase of 3.0 °C (GWSc-2), the temperature conditions in Rome, Tirana and Larnaca will lead to a very high risk (> 0.1) with spoilage probabilities of 0.17, 0.12 and 0.20, respectively. For the latter scenario a high risk (10-3 - 10-1) is predicted for Belgrade (3.8 x 10-2), Bucharest (1.4 x 10-2) and Athens (5.7 x 10-2), while for Chisinau and Valetta the predicted risk is 1.3 x 10-4 and 2.1 x 10-4, respectively. For the extreme scenario of 4.5 °C temperature increase (GWSc-3), a very high risk is predicted for 7 cities (Belgrade, Bucharest, Rome, Tirana, Athens, Valetta and Larnaca), a high risk for 7 cities (Bratislava, Chisinau, Budapest, Zagreb, Sarajevo, Sofia and Lisbon) and a medium risk (10-6 - 10-3) in 3 cities (Maribor, Paris and Vienna).

The above simulation results for canned milk risk of spoilage demonstrate that a temperature increase above 2 °C due to climate change, (GWSc-2 and GWSc-3) will lead to an increased number of spoilage events during delivering shelf-stable foods at the sales points of the Southern European region. Considering that the contamination of shelf-stable foods with thermophilic spores cannot be eliminated, the latter is expected to force the food business operators to major adjustments of their logistics. Shelf-stable foods are currently distributed and stored at ambient conditions using non-insulated trucks, shipping containers and storage rooms. The most effective strategy for the food industry to mitigate the increased risk of spoilage due to climate change would be to distribute and store shelf-stable foods under refrigeration, following the same logistic routes of perishable foods. The later however, would have a huge impact on the energy cost related to food transportation and storage. Indeed, refrigeration of foods is one of the most energy-intensive technologies used in the food supply chain, accounting for about 35% of the total energy consumption in the food industry worldwide (Evans et al., 2014; Tassou et al., 2009). In addition, food refrigeration has a high contribution to greenhouse gas emissions, accounting for approximately 1% of global CO_2 emission (James and James, 2010). Moreover, most refrigerants also split and release ozone destructive chlorine atoms, leading to increased harmful ultraviolet radiation reaching the ground (Tassou et al., 2009; Wu et al., 2013). Consequently, the option of delivering shelf-stable food under refrigeration poses a number of significant environmental and sustainability related risks.

The risk assessment model shows that the high risk of spoilage is mainly related to the extensive microbial growth that occurs during the hottest hours of the summer days when foods are exposed for several hours to high temperatures above the minimum temperature for growth.

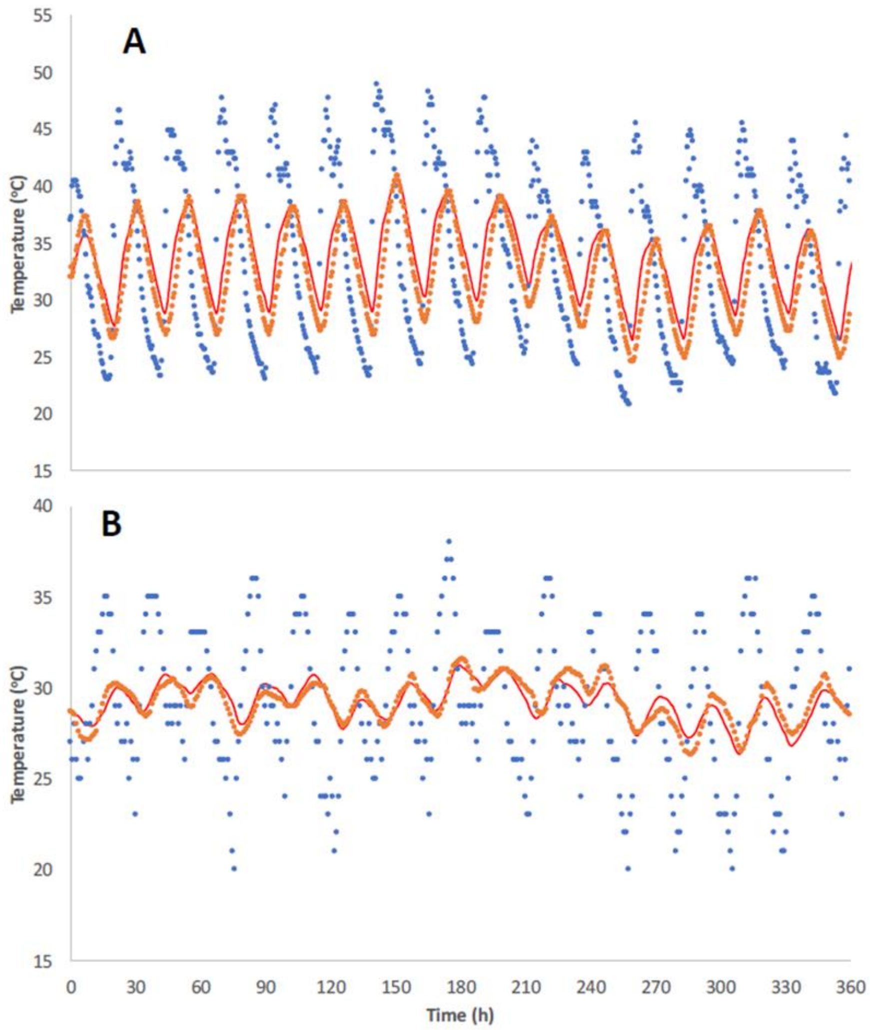


Figure A6. Representative fitting of insulated transportation and storage. **A**: Insulated truck **B**: Insulated storage room inner temperature using an exponential smoothing model (Eq. (5.8)). Blue points: recorded external temperature, orange points: recorded inner temperature, red line: fitted inner temperature.

Based on the above observation, an alternative to refrigeration mitigation strategy could be the non-refrigerated but insulated transportation and storage of shelf-stable foods, which can significantly decrease the maximum temperatures that the products are exposed. **Figure A7** shows a comparison of representative ambient external air temperature profiles with inner air temperature of non-insulated and insulated truck container and storage room of shelf stable foods. As shown in the latter figure, temperature evolution during non-insulated storage is very close to the ambient temperature, while insulated storage results in a significant decrease of the maximum temperature. To assess the impact of insulated transportation and storage on the risk of spoilage, we rerun the risk assessment model for canned milk with adjusting the annual collected temperature profiles for the 38 European cities to insulated transportation and storage using an exponential smoothing model (**Figure A6**). The simulation results show that insulation eliminates the risk of spoilage in all tested cities for the scenarios of 1.5 °C (GWSc-1) and 3 °C (GWSc-2) increase. For the extreme scenario of 4.5 °C temperature increase (GWSc-3), the risk was eliminated for all cities except from Rome and Larnaca. However, the probability of spoilage is 75 and 18 times lower, respectively, compared to non-insulated storage.

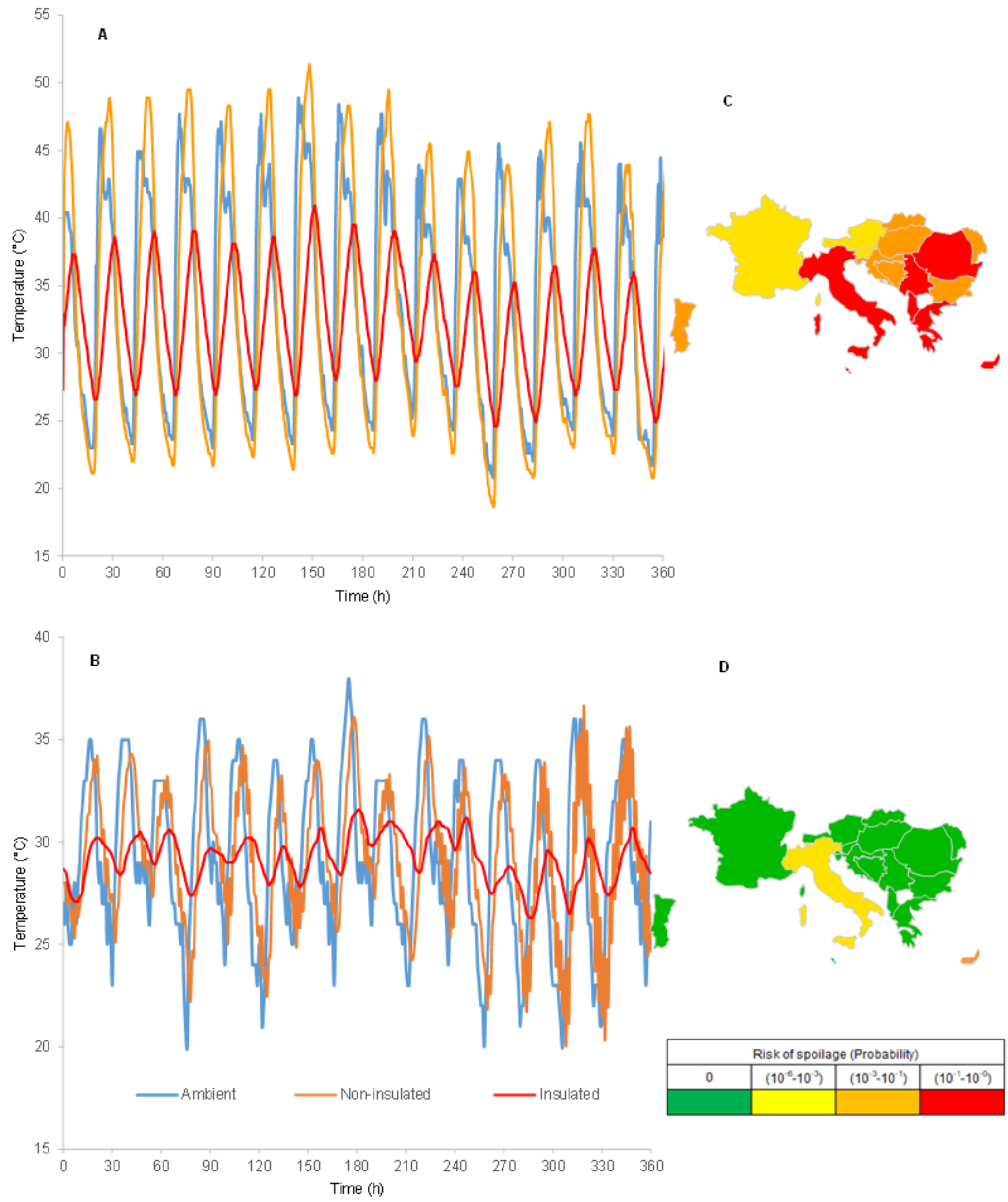


Figure A7. Insulated transportation and storage as a risk mitigation strategy. **A**: Representative profiles for ambient (outdoor) and indoor temperature of non-insulated and insulated food transportation truck. **B**: Representative profiles for ambient (outdoor) and indoor temperature of non-insulated and insulated storage room. **C**: Predicted risk of spoilage for GWSc-3 assuming non-insulated storage. **D**: Predicted risk of spoilage for GWSc-3 assuming insulated storage.

Conclusions

Our findings evidence that the current temperature conditions during distribution and storage are marginal in controlling the growth of thermophilic spoilage bacteria in shelf-stable foods. Through a risk assessment model, we demonstrated that a temperature increase above 2 °C, will lead to an increased number of spoilage events during distribution of shelf-stable foods in the Southern European region. The effect of insulated transportation and storage as a mitigation strategy to reduce the high risk of spoilage expected from global warming was also investigated and led to the elimination of the spoilage risk for the temperature increase scenarios of 1.5 °C (GWSc-1) and 3 °C (GWSc-2) while significantly reducing the risk of spoilage for the extreme scenario of 4.5 °C temperature increase (GWSc-3). The results of the present study can be considered as an alert for the expected impact of climate change on the microbiological stability of shelf-stable foods and stress the need for a high level of preparedness by both the food industry and policy makers.

Case study B: Geobacillus stearothermophilus in plant-based milk alternatives

Background

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 sterilization 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. 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 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 (IPPC, 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 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. 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 exceed its maximum concentration (*Nmax*) at the time of consumption. The maximum concentration of *G. stearothermophilus* in PBMA 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.

Materials and Methods

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.

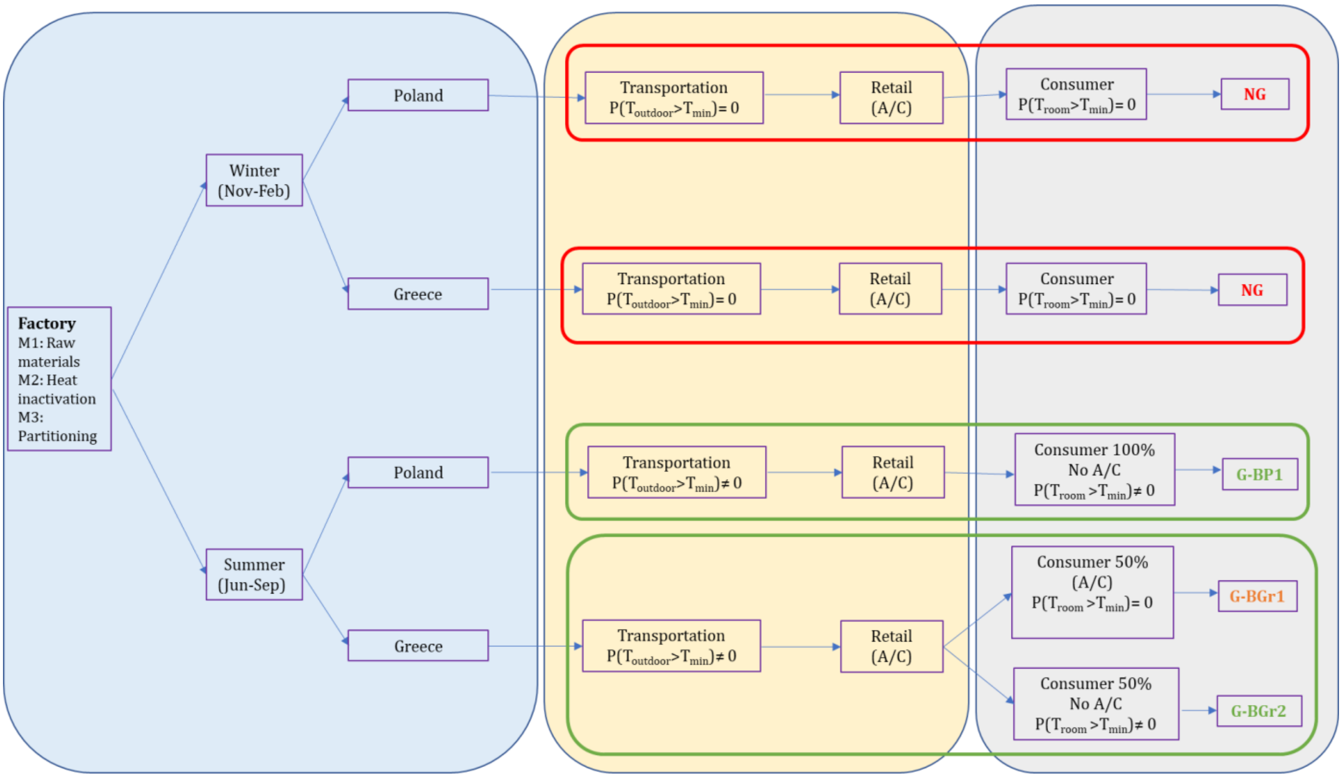


Figure B1. Decision tree of the Quantitative Microbial Spoilage Risk Assessment (QMRSA) of plant-based milk alternatives by Geobacillus stearothermophilus in Europe. In the baseline scenario the production in Poland and Greece is split into two periods, namely winter and summer season. During winter 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).

Module 1: Raw materials

The initial contamination level in the raw materials were obtained from the literature (NIZO, IAFP, 2022). A total thermophilic bacteria load up to 10,000 CFU/g for the different tested plant 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) (Eq.9)

prevalence ~Beta (s+1, n-s+1) (Eq.10)

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; Most likely; Max) (Eq. 11)

With:

Min ~Uniform (0;1) (Eq.12)

Most likely ~Uniform (1;3) (Eq.13)

Max ~Uniform (3;5) (Eq.14)

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

$$N_{0_t} = N_{0_{rm}} \times V_t \quad \text{(Eq. 15)}$$

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. The obtained D-values were transformed using a $\log_{10}$ transformation and plotted against temperature (**Figure B2**).

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:

$$log_{10}D = log_{10}D_{ref} + \frac{(T_{ref} - T)}{z} + \varepsilon_1 \quad \text{(Eq. 16)}$$

$$\varepsilon_1 \sim Normal(0, sd_1) \quad \text{(Eq. 17)}$$

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 representative of variability due to the different strains included in the D-values dataset. Uncertainty was not taking into consideration due to the significant amount of data.

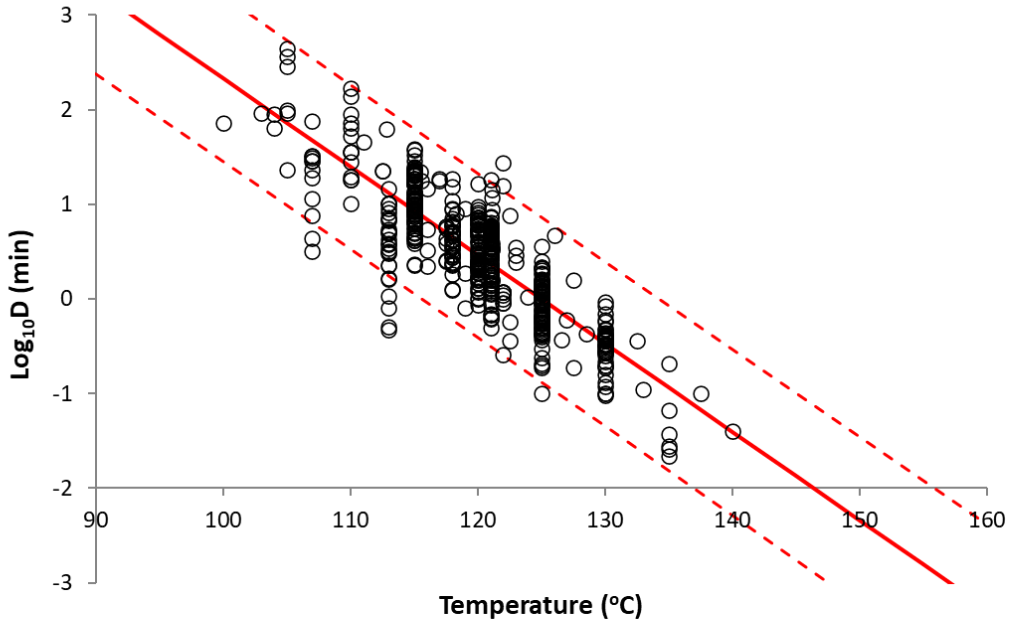


Figure B2. Effect of temperature on the decimal reduction time (D) of Geobacillus stearothermophilus. Points represent the logarithm of the 566 obtained D-values (log10D), and the red solid line indicates the fitting of the secondary model to the data.

Since UHT treatment is usually applied in different time-temperature combinations, a scenario at temperature 140 °C for 3 sec was generated for the heat treatment module. The number of spores surviving the UHT treatment (N_{HT}) was estimated using a Poisson distribution, as suggested by Nauta et al., 2001 and recently used by Santos et al., 2020:

$$N_{HT} \sim Poisson\left(N_{0_t} \times Pr_{sur}\right) \quad \text{(Eq. 18)}$$

$$Pr_{sur} = 10^{\left(\frac{-t_{HT}}{D}\right)} \quad \text{(Eq. 19)}$$

Where:

N_{0_t} is the number of spores present in the raw material per tank, as defined in previously and Pr_{sur} is the probability for one spore to survive the heat treatment (Nauta et al., 2001) deduced from Eq. 16.

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:

$$N_p \sim Poisson\left(\frac{N_{HT}}{V_t} * V_p\right) \quad \text{(Eq. 20)}$$

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 **Figure B1**). 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). 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:

$$t_{tr} \sim Uniform\ (2; 12) \qquad \text{(Eq. 21)}$$

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 and did not allow growth. The percentage of insulated truck was described through a Bernoulli distribution with Uniform distribution capturing the uncertainty as follows:

$$\text{Bernoulli}\ (1, P_{truck}) \qquad \text{(Eq. 22)}$$

$$P_{truck} \sim Uniform(0.1; 0.5) \qquad \text{(Eq. 23)}$$

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.

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:

$$\mu_{max} = \mu_{opt} * \gamma(T) * \gamma(pH) \qquad \text{(Eq. 24)}$$

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:

$$\gamma(T) = \frac{(T-T_{min})^2(T-T_{max})}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]} + \varepsilon_2 \qquad \text{(Eq. 25)}$$

$$\varepsilon_2 \sim Normal(0, sd_2) \qquad \text{(Eq. 26)}$$

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:

$$\gamma(pH) = \frac{(pH-pH_{min})(pH-pH_{max})}{(pH_{opt}-pH_{min})(pH-pH_{opt})-(pH_{opt}-pH_{max})(pH_{min}-pH)} + \varepsilon_3 \qquad \text{(Eq. 27)}$$

$$\varepsilon_3 \sim Normal(0, sd_3) \qquad \text{(Eq. 28)}$$

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 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. 29-31).

$$N_{trans} = N_p * \exp(\mu_{max} * t_{trans}) \qquad \text{(Eq. 29)}$$

Where:

μ_{max} is derived from Eq. 16 with $T=T_{tr}$

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

$$N_{night} = N_{trans} * \exp(\mu_{max} * t_{night}) \qquad \text{(Eq. 30)}$$

Where:

μ_{max} is derived from Eq. 16 with $T=T_n$

$$N_f = N_{night} * \exp(\mu_{max} * t_{day}) \qquad \text{(Eq. 31)}$$

Where:

μ_{max} is derived from Eq. 24 with $T=T_d$

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

$$C_{final} = \frac{N_f}{250} \quad \text{(Eq. 32)}$$

Model implementation

The spoilage risk assessment model was implemented in R software (R Core Team, 2019). 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.

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.

Climate change scenario

A climate change scenario (CCs) was designed, based on the projections provided by the IPCC (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.

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.

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.

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.

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. 10 with V & U separated	NIZO, 2022
Microbial concentration in raw materials	$N_{0_{rm}}$	Log CFU/mL	Pert (Min; Most likely; Max)	Eq. 11 with V & U separated	NIZO, 2022
Volume of the tank	V_t	L	1000	Eq. 15 fixed	This study
Module 2 : Heat-treatment					
Reference temperature	T_{ref}	°C	121	Eq. 16 fixed	This study
Decimal reduction time reference	D_{ref}	min	2.34	Eq. 16 fixed	Deduce from fitting D-values (Sup. data)
Temperature resistance	z	°C	10.70	Eq. 16 fixed	Fitting D-values (Sup. data)
Heat inactivation secondary model error (Eq.9)	sd_1	Log min	0.35	Eq. 17 V	Fitting D-values (Sup. data)
Time of the treatment	t_{HT}	sec	Uniform (3;9)	Eq. 16 V	This study
Temperature of the treatment	T_{HT}	°C	Uniform (130;150)	Eq. 16 V	This study
Module 3 : Partitioning					
Volume of product unit, a pack	V_p	ml	250	Eq. 20 fixed	This study
Module 4 : Growth during distribution and storage					

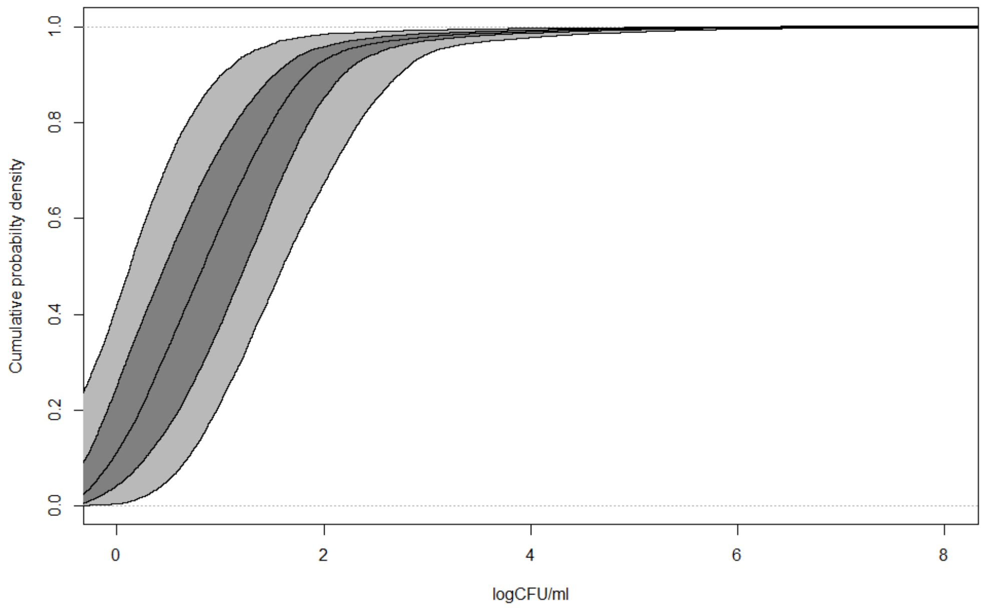
Minimum temperature of growth	T_{min}	°C	33.76	Eq. 25 fixed	Kakagianni et al., 2016
Optimum temperature of growth	T_{opt}	°C	61.82	Eq. 25 fixed	Kakagianni et al., 2016
Maximum temperature of growth	T_{max}	°C	68.14	Eq. 25 fixed	Kakagianni et al., 2016
Secondary cardinal temperature model error	sd_2	-	0.003	Eq. 26 U	Kakagianni et al., 2016
Optimum growth rate	μ_{opt}	h^{-1}	Empirical distribution based on μ_{opt} data & bootstrap	Eq. 24 with V & U mixed	Misiou et al., 2021
Minimum pH of growth	pH_{min}	-	5.65	Eq. 27 fixed	Misiou et al., 2021
Optimum pH of growth	pH_{opt}	-	6.74	Eq. 27 fixed	Misiou et al., 2021
Maximum pH of growth	pH_{max}	-	8.71	Eq. 27 fixed	Misiou et al., 2021
pH of the product	pH	-	7.00	Eq. 27 fixed	This study
Secondary cardinal pH model error	sd_3	-	0.056	Eq. 27 U	Misiou et al., 2021
Temperature during distribution	T_{tr}	° C	Empirical distribution based on T dataset	Eq. 25 V	Data loggers Sup. data
Time during distribution	t_{tr}	h	Uniform (2;12)	Eq. 21 V Eq. 29	This study
Percentage of insulated trucks	P_{truck}	-	Uniform (0.1;0.5)	Eq. 22 with V & U separated	This study

Temperature of the product storage-retail	T_r	°C	Below 25	Eq. 25 fixed	This study
Temperature of the product storage during the day at consumer place	T_d	°C	Empirical distribution based on T dataset	Eq. 25 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. 25 V	www.wunderground.com
Time of the product night-consumer	t_n	days	60	Eq. 30 fixed	This study
Time of the product storage day-consumer	t_d	days	60	Eq. 31 fixed	This study

Results and Discussion

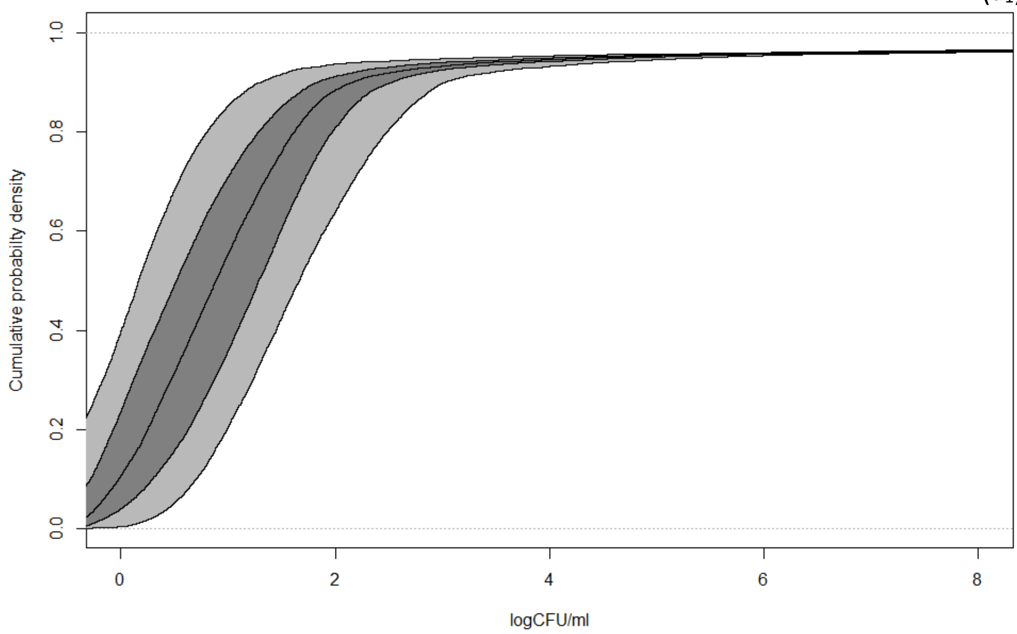
Level of Geobacillus stearothermophilus in plant-based in Europe and associated risk of spoilage under the current climatic conditions

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 **Figure B3a** and **B3b**. 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) (**Figure B3a$_1$**) and 2.5 (1.9; 3.3) (**Figure B3a$_2$**), when an A/C system was assumed to be in place.



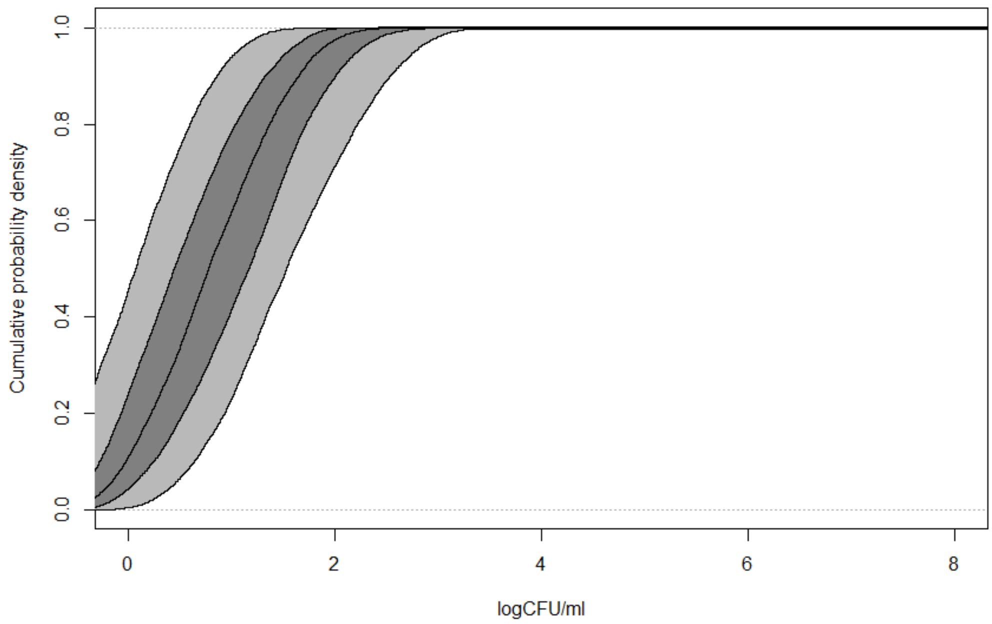
Cumulative probability density
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
logCFU/ml

(a$_1$)



Cumulative probability density
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
logCFU/ml

(a$_2$)



(b)

Figure B3. 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 25^{th} and 75^{th} percentiles of the uncertainty.

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 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})).

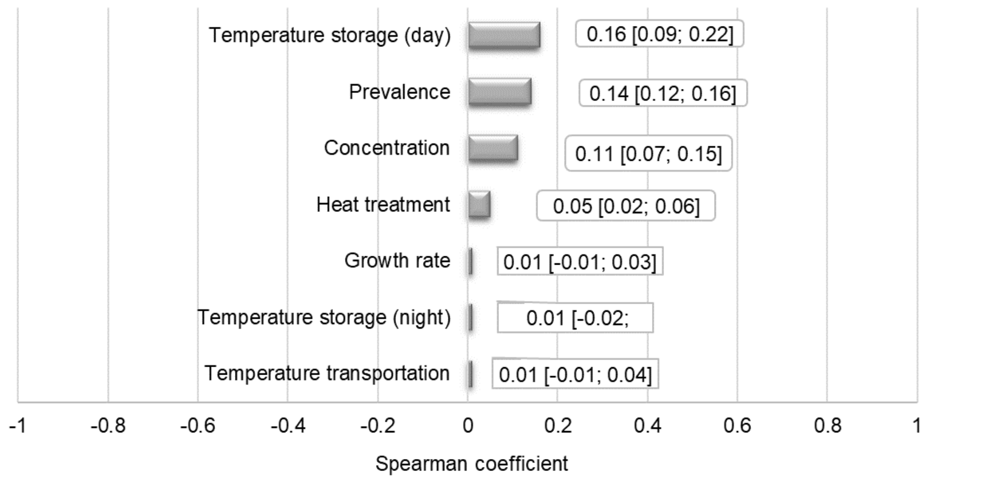
Table 2. Risk of spoilage due to growth of *Geobacillus stearothermophilus* in plant-based milk alternatives distributed and stored in North and South Europe under the current climatic conditions.

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}

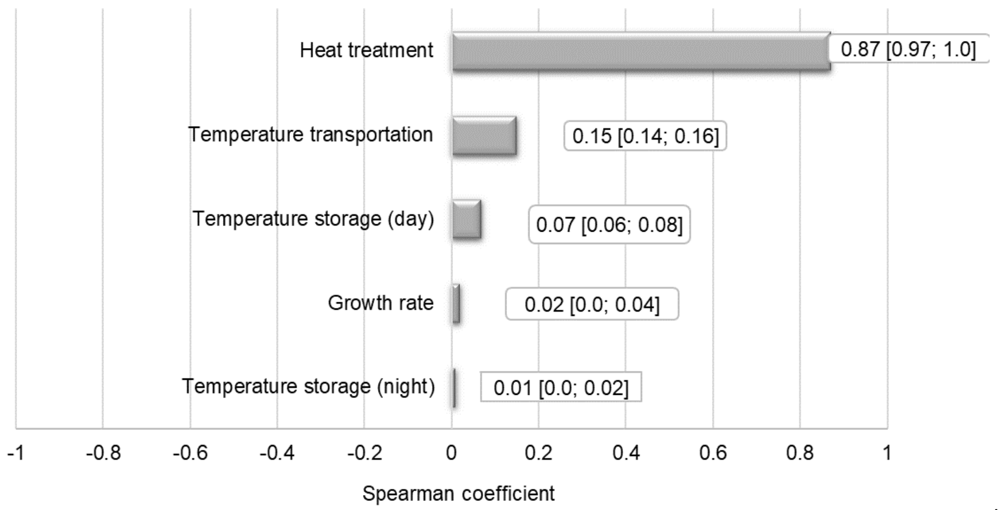
Influence of model inputs on the estimation of the current risk

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 **Figure B4a** 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 (**Figure B4a**). 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 **Figure B4b**. The variability related to the heat treatment has the highest impact (0.87 95% CI (0.97; 1.00)) 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 (0.97; 1.00). 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.



(a)



(b)

Figure B4. Tornado plot illustrating the sensitivity analysis of all the variable inputs on the risk of spoilage **(a)** and on the final concentration (LogN) (**b**) of *Geobacillus stearothermophilus* in plant-based milk alternatives in Greece during summertime. Spearman coefficient estimates with 95% uncertainty interval in bracket.

Prediction of spoilage risk under a 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.

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]	1.98 (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.

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 either for 3 or 7 sec failed to significantly reduce the risk 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 not significantly decrease the risk of spoilage. The spoilage risk was estimated to be only significantly reduced 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.

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)	1.02.0.99; 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)

Conclusions

To the best of our knowledge, this is the first attempt to employ a 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 Pujot and colleagues (Pujot 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 (Koutsouamanis 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 uncertainty associated with the assumptions made along with the data used in this model could affect the estimation of the risk. The latter is of a great importance especially when the goal of the QMRA is to estimate the absolute risk (EFSA, 2014). A 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 log10-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).

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 over-estimated, 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 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 quantifying 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.

Climate Change Scenarios

Background

The IPCC is the United Nations body that serves as the link between the scientific community and the policy makers regarding climate change. The IPCC releases regular scientific assessment reports about state-of-the-art knowledge. The second and third chapters of the Third Assessment Report (TAR) of the IPPC, which was published in 2001, introduced the methodology followed to conduct impact assessments and develop climate change scenarios, respectively. Since then, the common practice to assess the impact of climate change on the food sector is to conduct a climate change impact assessment. This methodology utilizes climate models along with impact models (Katsini et al., 2021) and has already been employed in multiple cases related to food security that are summarized in the chapter titled food security of the "Climate Change and Land" report published by the IPCC in 2019 (IPCC, 2019). Such studies deal mainly with crops such as cereals (Guan et al., 2017), maize (Warnatzsch et al., 2020; Zizinga et al., 2022; Bocchiola et al., 2013; Chung et al., 2014), wheat (Wang et al., 2018), as well as rice (Wang et al., 2014; 2017; Zheng et al., 2020; Poulton et al., 2016; Islam et al., 2020; Wu et al., 2021), fisheries (Dey et al., 2016; Yakubu et al., 2022), beans (Antolin et al., 2021) and soybean (da Silva et al., 2021). Compared to food security, very limited research can be found in the context of food safety. Battilani and colleagues (2016) deal with mycotoxins in maize in Europe, and Van der Fels-Klerx and colleagues (2013) focus on mycotoxins in cereal grains in the Netherlands. Galvao et al. (2021) focus on mercury from fish in Brazil, and Pedersen et al. (2014) on parasites from snails in Zimbabwe. Ndraha et al. (2019) implement the impact modelling framework for Vibrio parahaemolyticus in raw oysters in Taiwan, while Liu et al. (2015) present a toy example for Lactobacillus plantarum in Belgium. A crucial step when conducting such an analysis is preparing suitable climate change projections along with a rigorous uncertainty analysis (IPCC, 2001), which is the focus here.

In general, climate modelling has two goals: understanding the climate system and yielding climate projections for the future, which is the aim here. These are produced by taking into account a variety of prospective future climate change mitigation scenarios. Every assessment report builds up on the previously used scenarios and presents a new set. For example, the fifth assessment report (IPCC, 2013) used the representative concentration pathways, while the sixth (IPCC, 2021) the Shared Socioeconomic Pathways (SSPs). SSPs are a set of different feasible trajectories of societal development that are based on hypotheses regarding the societal components that are the major factors affecting the challenges in mitigating and adapting to climate change (O'Neill et al., 2014). Such factors are population growth, economic growth, education, urbanization, and the rate of technological development. The scenarios are (**Figure C1**): SSP1: Sustainability (Taking the Green Road), SSP2: Middle of the Road, SSP3: Regional Rivalry (A Rocky Road), SSP4: Inequality (A Road divided), and SSP5: Fossil-fueled Development (Taking the Highway).

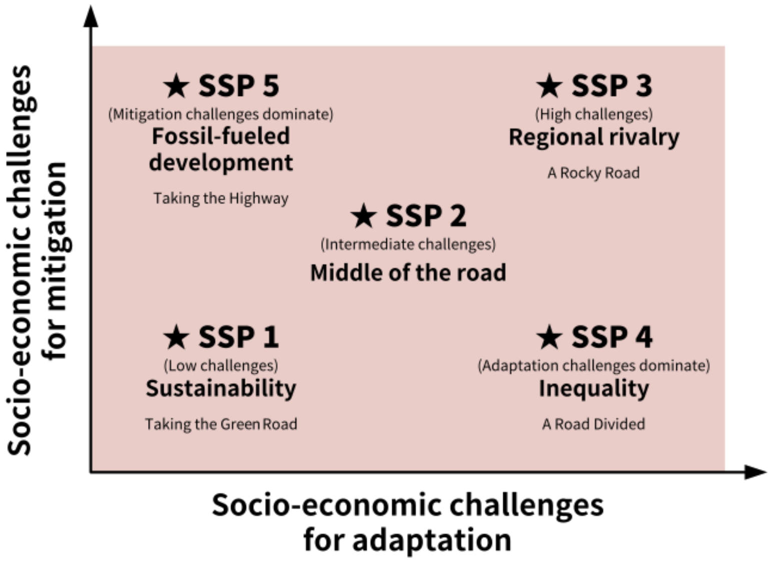


Figure C1. Overview of the SSPs (O'Neill et al., 2014).

Similar to the climate change mitigation scenarios, the climate models have progressed since their initial development. Climate models are mathematical models that simulate the Earth's atmosphere along with the rest of the climate compartments which are the hydrosphere, the cryosphere, the biosphere, and the lithosphere, and their interactions The Earth is divided into boxes, or grids, in the three spatial dimensions. These grids are all regarded as homogenous. The grid size of the climate model decreases as resolution increases (**Figure C2**). Climate scientists from around the world are working together as part of the Coupled Model Intercomparison Project (CMIP) of the World Climate Research Programme to share, evaluate, and compare developed climate models produced by various modelling organizations globally. When it comes to climate modelling, CMIP is thought to be state-of-the-art, and it is presently in its sixth phase (CMIP6) (Eyring et al., 2016).

Since no single model has been proven to be the most effective at modelling the climate system, an ensemble of models should be taken into account to conduct climate change impact assessments. The climate projections obtained from the climate models simulations include future trajectories of maximum, minimum, and average temperature, as well as other climate variables, such as precipitation and wind speed.

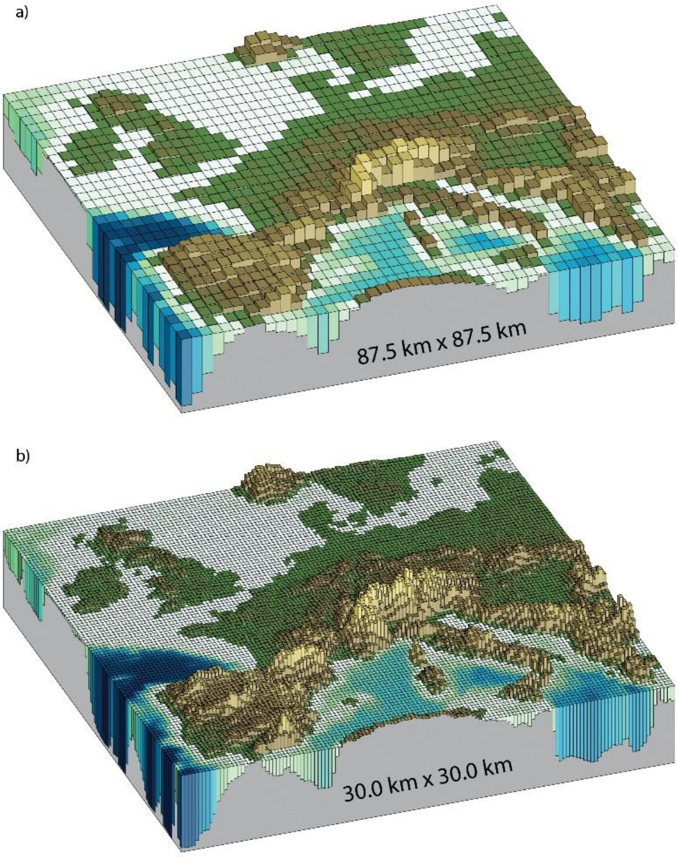


Figure C2. Illustration of the European topography at: (a) resolution of 87.5 × 87.5 km; (b) same as (a) but for a resolution of 30.0 × 30.0 km. (IPCC, 2013).

Across all the published IPCC reports the uncertainty analysis comprises an important aspect. The main sources of uncertainty in climate projections are (1) scenario uncertainty, which, in this case, is related to the variability among the SSPs, (2) model uncertainty, which originates from the climate models and is linked to parametric and other structural uncertainties, and (3) internal variability, referring to the natural climate variability (IPCC, 2021).

Material and Methods

In this tutorial, the aim is to prepare suitable climate change projections for a climate change impact assessment concerning the microbial food safety of raw milk produced in farms located in the Maltese islands. This imposes a set of constraints for the climate projections: they should have a daily temporal resolution, and the Maltese islands need to be covered by at least two grid boxes of the climate models that these projections come from. We use the most up-to-date tools available, thus, we consider only CMIP6 models and SSPs. The workflow can be broken down into two parts: one for obtaining the proper climate projections that fulfill the requirements of our case study, and one for partitioning the different sources of uncertainty in our regional CMIP6 climate projections. The methodology for the second part is analyzed at the end of this sub-section.

Obtaining Climate Projections

The CMIP6 climate projections are accessed through the climate data store of the Copernicus Climate Chane Service (C3S), operated by the European Centre for Medium-Range Weather Forecasts (ECMWF) on behalf of the European Union (C3S, 2022). The available SSPs that meet the requirements are SSP1, SSP2, SSP3, and SSP5. The available climate variables with a daily frequency corresponding to the CMIP6 climate models are:

• Near-Surface air temperature [K]: Temperature of air at 2m above the surface of land, sea or inland waters. 2m temperature is calculated by interpolating between the lowest model level and the Earth's surface, taking account of the atmospheric conditions.

• Daily maximum near-surface air temperature [K]: Daily maximum temperature of air at 2m above the surface of land, sea or inland waters.

• Daily minimum near-surface air temperature [K]: Daily minimum temperature of air at 2m above the surface of land, sea or inland waters.

• Near-surface specific humidity [-]: Amount of moisture in the air near the surface divided by amount of air plus moisture at that location.

• Near-surface wind speed [m/s]: Magnitude of the two-dimensional horizontal air velocity near the surface.

• Precipitation [kg/m^2s]: The sum of liquid and frozen water, comprising rain and snow, that falls to the Earth's surface. It is the sum of large-scale precipitation and convective precipitation. This parameter does not include fog, dew or the precipitation that evaporates in the atmosphere before it lands at the surface of the Earth. This variable represents amount of water per unit area and time.

The climate models that pass the screening for the temporal requirements are listed below. The country in brackets is the country on which the modelling group which developed the model is based.

- ACCESS-CM2 (Australia)
- ACCESS-ESM1-5 (Australia)
- AWI-CM-1-1-MR (Germany)
- AWI-ESM-1-1-LR (Germany)
- BCC-CSM2-MR (China)
- BCC-ESM1 (China)
- CAMS-CSM1-0 (China)
- CanESM5 (Canada)
- CESM2 (USA)
- CESM2-FV2 (USA)
- CESM2-WACCM (USA)
- CESM2-WACCM-FV2 (USA)
- CMCC-CM2-HR4 (Italy)
- CMCC-CM2-SR5 (Italy)
- CMCC-ESM2 (Italy)
- CNRM-CM6-1 (France)
- CNRM-CM6-1-HR (France)
- CNRM-ESM2-1 (France)
- FGOALS-g3 (China)
- GFDL-ESM4 (USA)
- GISS-E2-1-G (USA)
- HadGEM3-GC31-LL (UK)
- HadGEM3-GC31-MM (UK)
- IITM-ESM (India)
- INM-CM4-8 (Russia)
- INM-CM5-0 (Russia)
- IPSL-CM5A2-INCA (France)
- IPSL-CM6A-LR (France)
- KACE-1-0-G (South Korea)
- KIOST-ESM (South Korea)
- MIROC6 (Japan)
- MIROC-ES2L (Japan)
- MPI-ESM-1-2-HAM (Switzerland)
- MPI-ESM1-2-HR (Germany)
- MPI-ESM1-2-LR (Germany)
- MRI-ESM2-0 (Japan)

- EC-Earth3 (Europe)
- EC-Earth3-AerChem (Europe)
- EC-Earth3-CC (Europe)
- EC-Earth3-Veg (Europe)
- EC-Earth3-Veg-LR (Europe)
- FGOALS-f3-L (China)
- NESM3 (China)
- NorCPM1 (Norway)
- NorESM2-LM (Norway)
- NorESM2-MM (Norway)
- SAM0-UNICON (South Korea)

The next step involves the spatial screening of these models so that the Maltese islands are covered by at least two grids. The resulting models are the ones comprising the multi-model ensemble, based on which the climate projections are estimated, and the uncertainty analysis is performed.

Partitioning Climate Projections Uncertainty

The methodology followed was introduced by Hawkins and Sutton (2009) and was revisited by Lehner and colleagues (2020). The total uncertainty (*T*) is the sum of the model uncertainty (*M*), the internal variability (*I*), and the scenario uncertainty (*S*). This assumes that the different sources are additive, which is not true as they are not orthogonal. The fractional uncertainty from a given source is then defined as *M/T, I/T*, and *S/T*. The first step is to estimate the forced response as a 4^{th}-order polynomial for each model and each SSP. Model uncertainty (*M*) is then computed as the SSP mean of the variance of the polynomial fits for each SSP. For the calculation of the internal variability (*I*), the climate projections are first smoothed using the running mean of one year. Afterward, the internal variability (*I*) for each model is defined as the variance over time of the residuals of the polynomial fits. Averaging across all models for each SSP, and then across all SSPs results in the multi-model mean internal variability (*I*), which is time-invariant. Finally, the scenario uncertainty (*S*) is defined as the variance across the multi-model means of the polynomial fits for the different SSPs.

Results and Discussion

To evaluate the climate models' grid positioning concerning the location of the Maltese islands, the temperature heat maps of all the climate models for a random date were constructed (**Figures C3, C4**, and **C5**). A heat map is a map that is colored based on the temperature values of the region under study. The climate models that pass the screening and will be part of the multi-model ensemble are listed below:

- BCC-CSM2-MR (China) 2 GRIDS
- CESM2 (USA) 2 GRIDS
- CESM2-WACCM (USA) 2 GRIDS
- CMCC-CM2-SR5 (Italy) 2 GRIDS
- CNRM-CM6-1-HR (France) 3 GRIDS
- GFDL-ESM4 (USA) 2 GRIDS
- INM-CM4-8 (Russia) 2 GRIDS
- IPSL-CM5A2-INCA (France) 2 GRIDS
- KIOST-ESM (South Korea) 2 GRIDS
- MRI-ESM2-0 (Japan) 2 GRIDS
- NorESM2-MM (Norway) 2 GRIDS

The 11 climate models yield the multi-model ensemble suitable for the Maltese islands on a daily temporal frequency that provides adequate climate change projections (**Figure C6**).

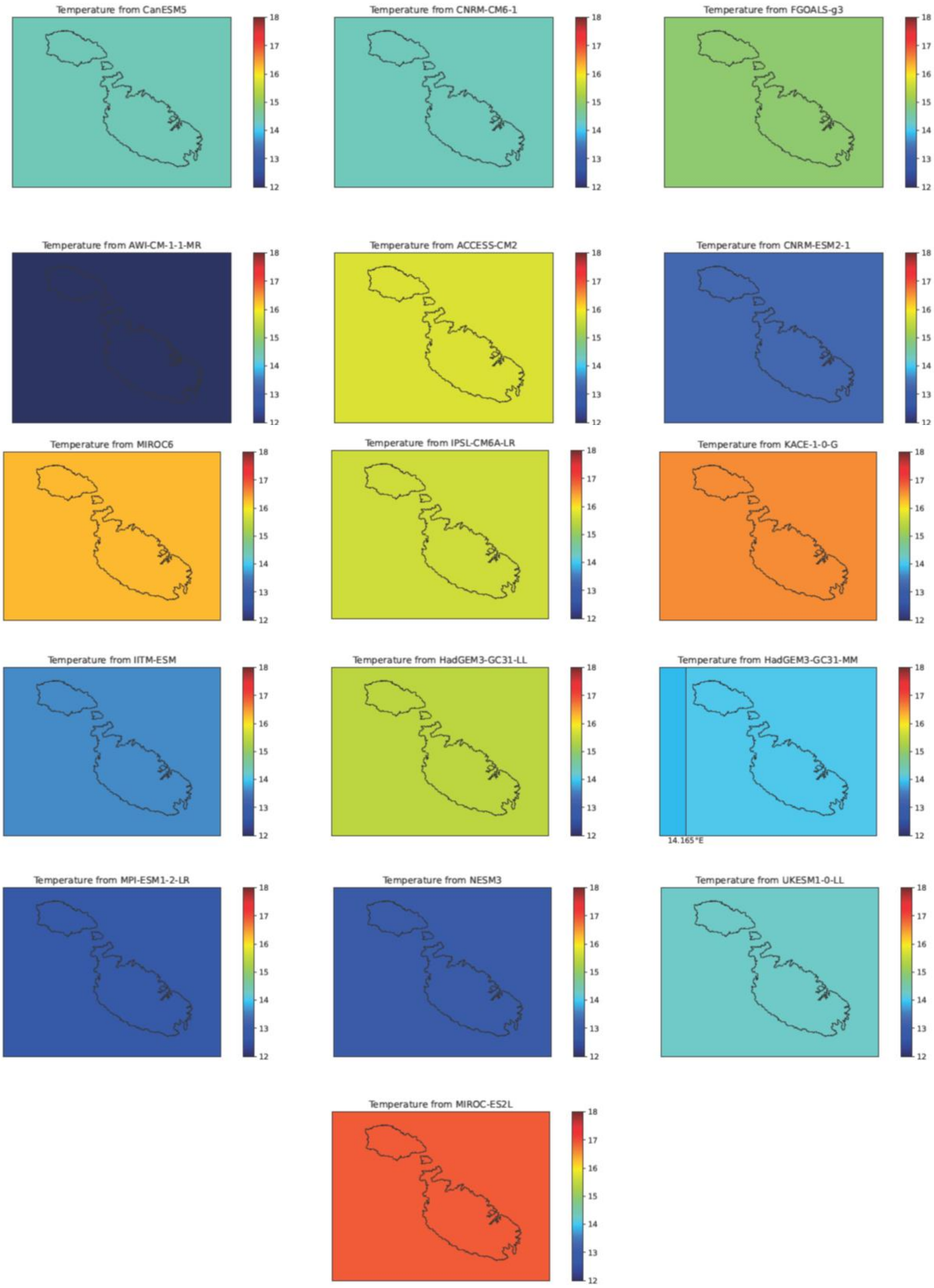


Figure C3. Heatmaps of climate models that use one grid to cover Malta

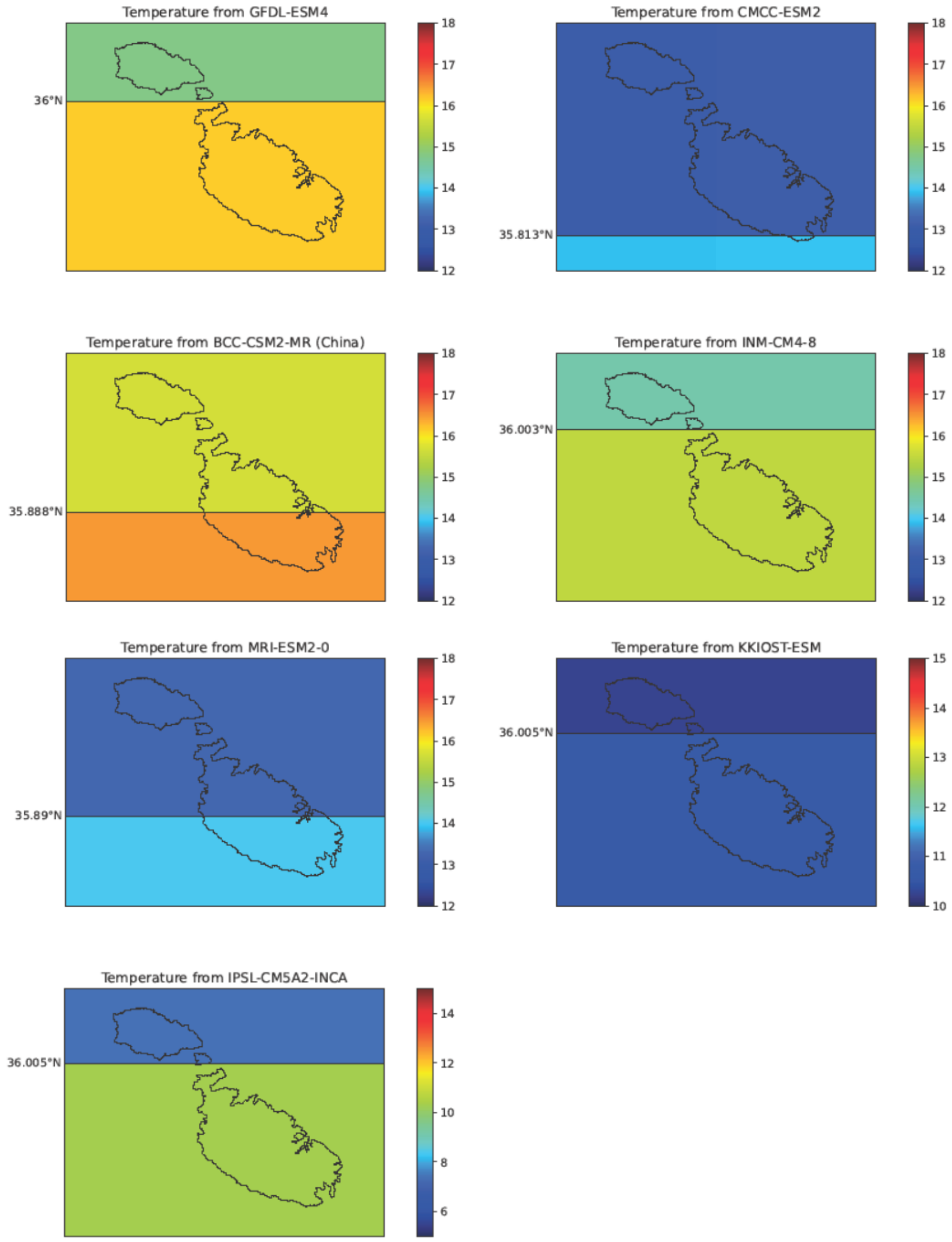


Figure C4. Heatmaps of climate models that use two grids to cover Malta. Even though model CMCC-ESM2 uses 2 grids, the coverage of the second one is too small to be considered two, thus it is considered to be misclassified.

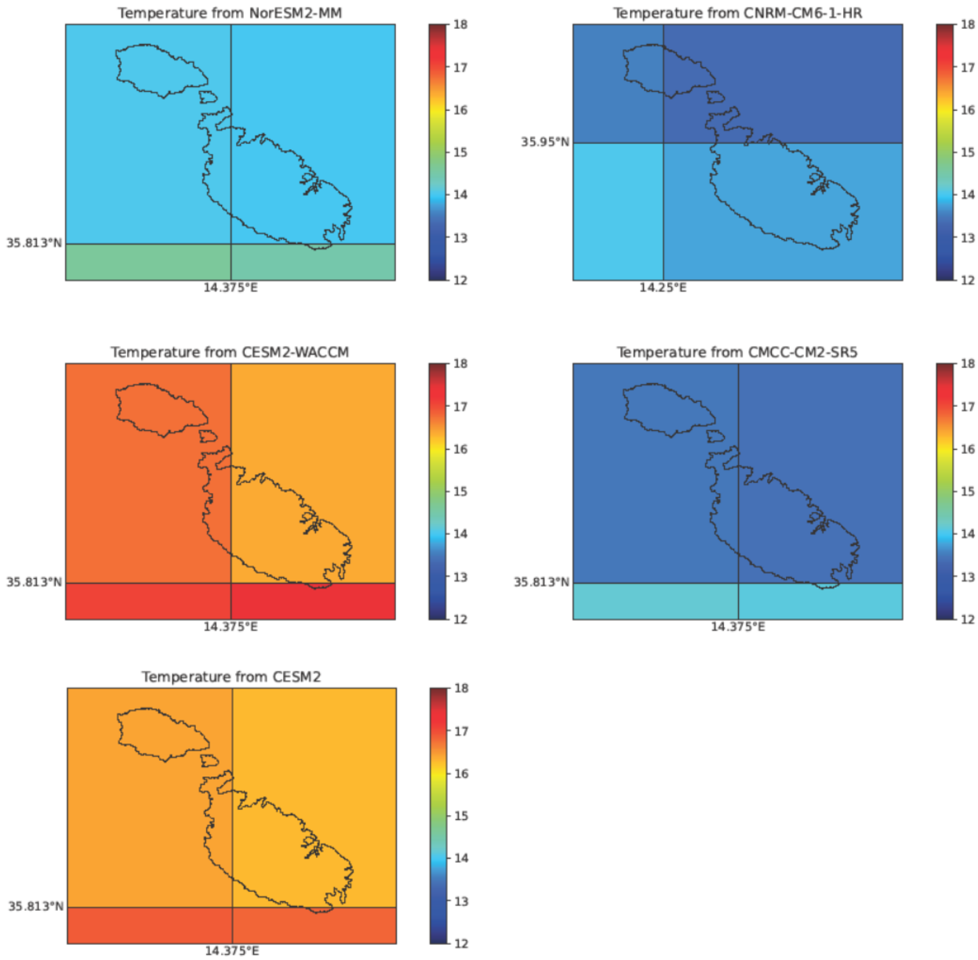
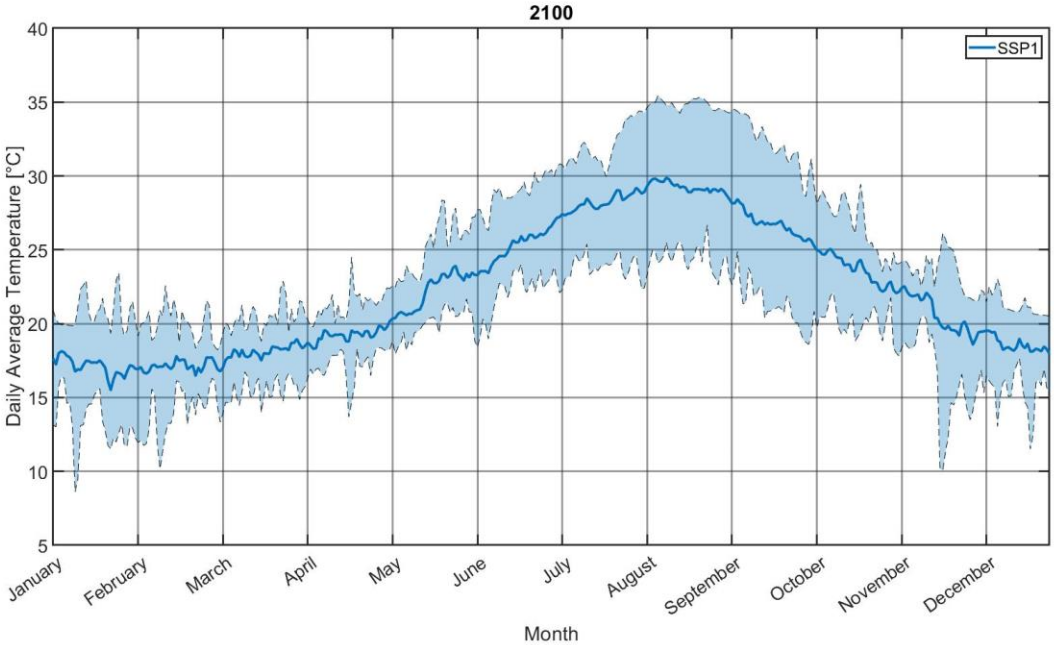


Figure C5. Heatmaps of climate models that use three grids to cover Malta. As we can see on the heatmaps above, all the models apart from model CNRM-CM6-1-HR, have in practice a 'two grid coverage' of the Maltese islands. CNRM-CM6-1-HR has a three-grid coverage.


2100
SSP1
Daily Average Temperature [°C]
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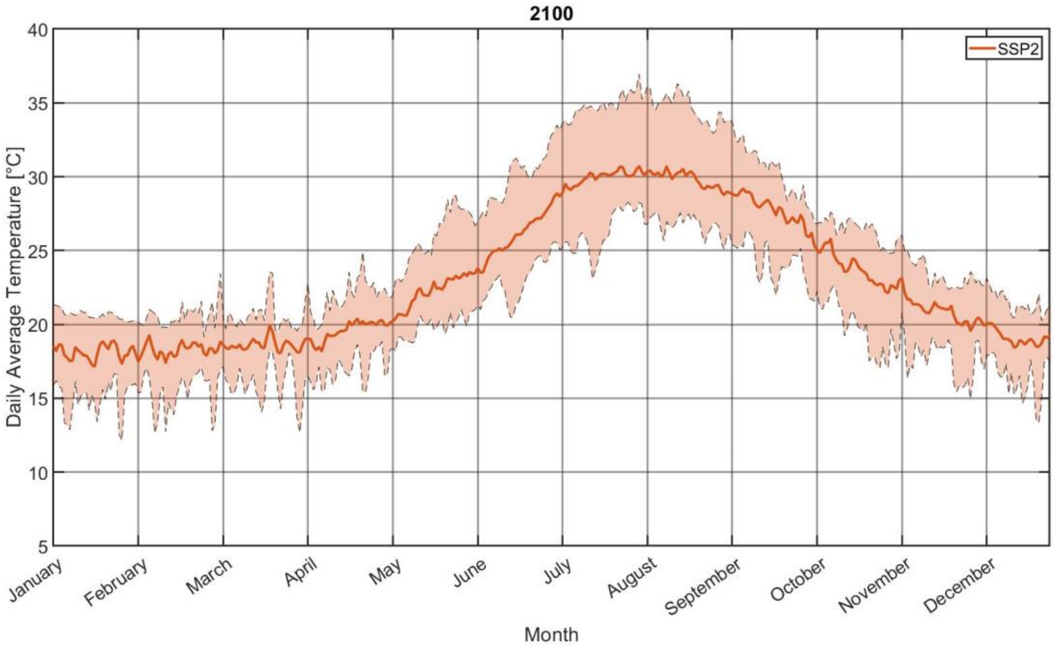
(a)


2100
SSP2
Daily Average Temperature [°C]
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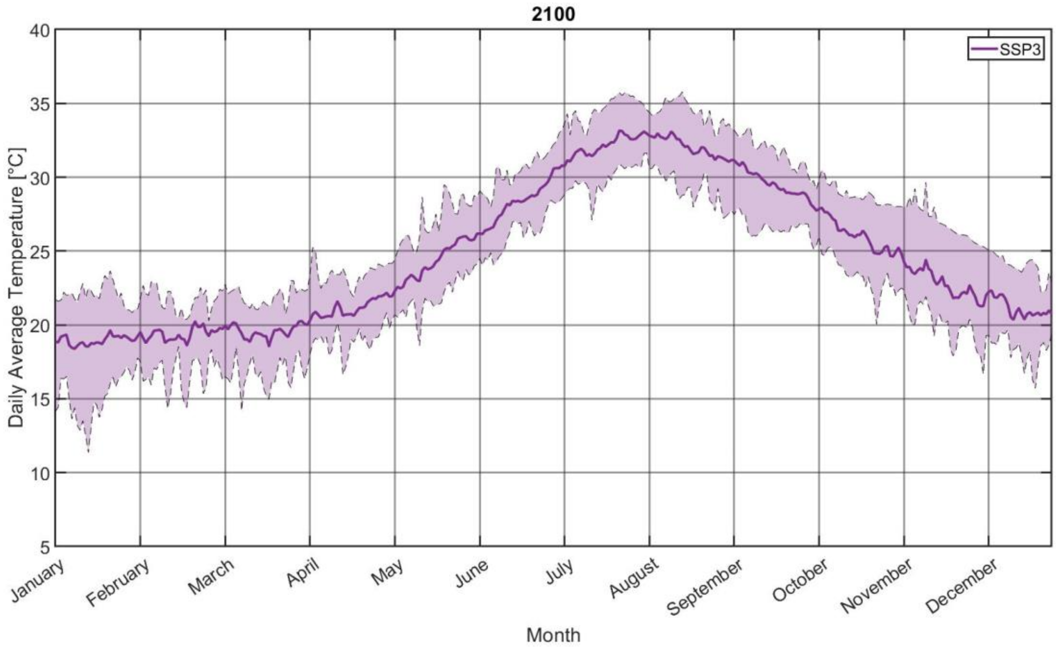
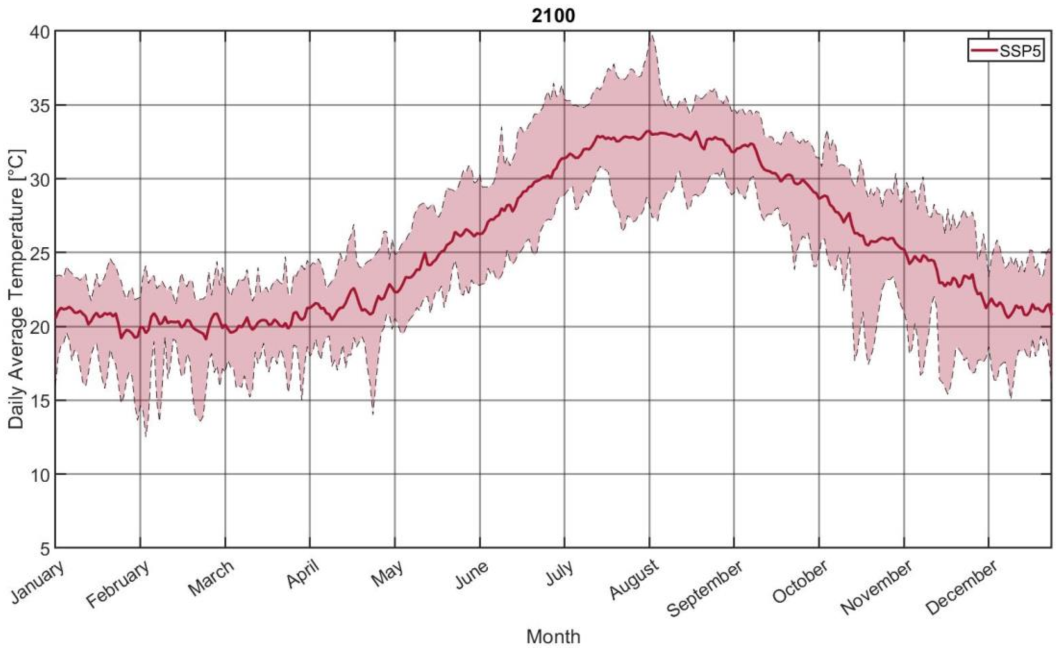
(b)



(c)



(d)

Figure C6. Daily average temperature climate change projections for 2100 considering SSP1 (a), SSP2 (b), SSP3 (c), and SSP5 (d). The uncertainty bounds shown are computed as the 90 percentile of the climate models' simulations.

The resulting polynomials used to compute the fractions of the different sources of uncertainty are presented in **Figure C6**. Results show that the projections corresponding to SSP3 and SSP5 in terms of multi-model mean are very close and start to divert after the year 2060. Furthermore, the spread of the models for SSP1 is considerably bigger compared to the spread of the models for the rest SSPs.

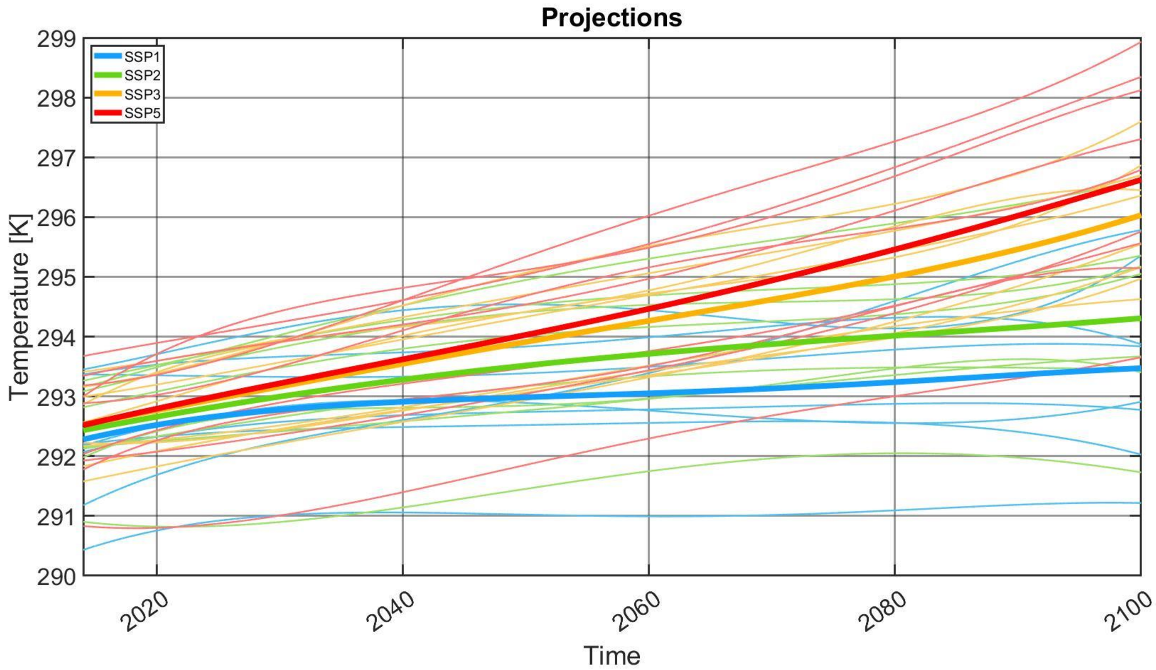


Figure C6. The polynomial fit for each CMIP6 model and the multi-model mean for each SSP.

The different sources of uncertainty are illustrated in two ways: in terms of the multi-model multi-scenario temporal projection (**Figure C7**) and in terms of fractional contribution of the individual sources to the total uncertainty (**Figure C8**).

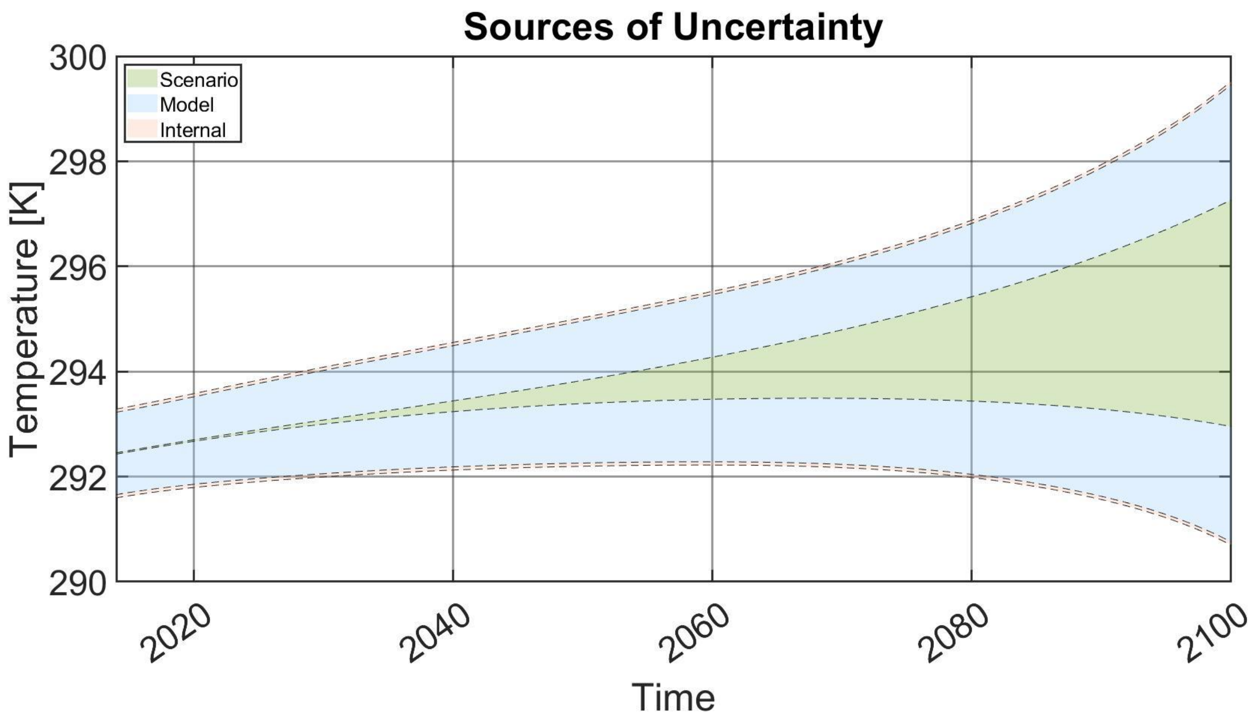


Figure C7. Illustration of the sources of uncertainty in the multi-model multi-scenario mean projection.

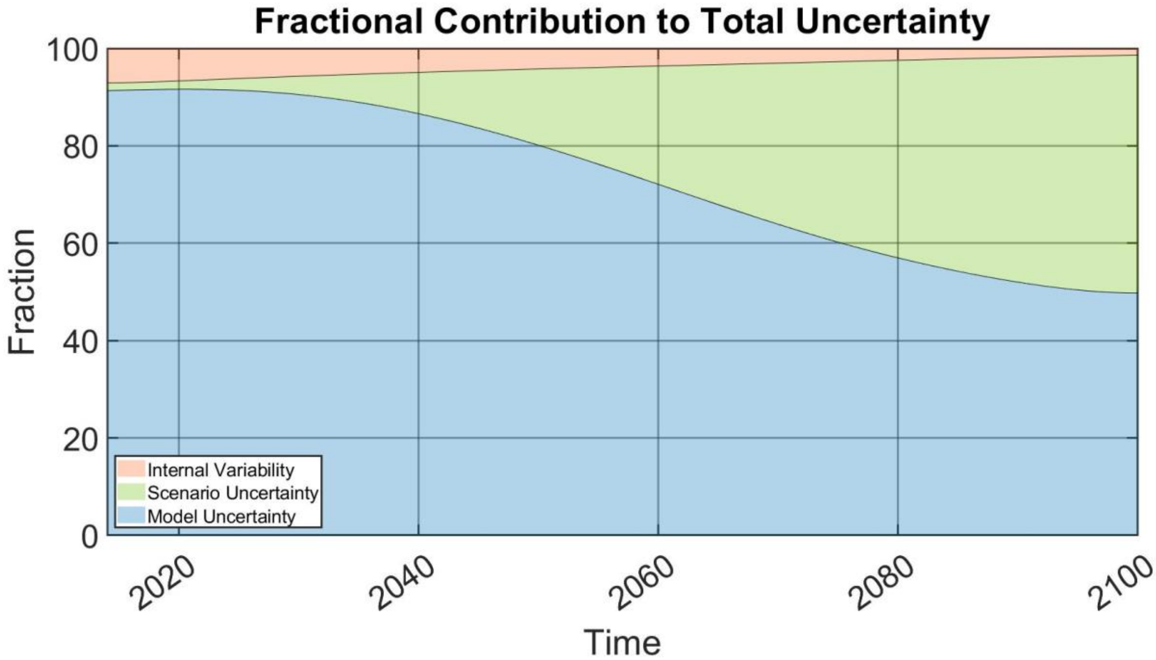


Figure C8. Illustration of the fractional contribution of the individual sources to total uncertainty with respect to projection horizon.

The results indicate that the model uncertainty is the biggest contributor. This means, that there is a potential to decrease the uncertainty in climate change scenarios by improving the climate models simulations for such fine scales. Moreover, as the prediction horizon increases, the total uncertainty increases. This can be explained by the fact that the influence of greenhouse gas emissions on the change of the climate is a phenomenon with delay, meaning that based on what is already emitted by this point, short-term climate change is predetermined. Furthermore, as the prediction horizon increases, the model uncertainty decreases and the scenario uncertainty increases. This can be attributed to the fact that the different SSPs are designed to lead to different possible futures, thus, should lead to a big range of future projections.

Conclusions

Climate change is one of the major drivers of change for food safety (FAO, 2022). Climate change's impact is more than increasing temperature, it includes altered weather patterns, and increased frequency and intensity of extreme weather events, such as heatwaves and droughts, ocean acidification, etc. (Tirado et al., 2010). The adequate methodology to quantitatively assess future food safety risks due to climate change is the impact modelling framework (IPCC, 2001) that enables the incorporation of these. In this section, we provide a tutorial by describing step-by-step the process of obtaining climate change projections. These are essential to conduct an impact assessment. Furthermore, the importance of attributing the uncertainty in the individual sources is underlined as it aids in planning for further research. This leads to a better understanding of the system, more accurate models, and thus, better interpretable results to facilitate evidence-based policymaking.

Acknowledgements

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