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Combined stochastic modelling of pathogenic and spoilage microorganisms

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Abstract

Quantitative microbiological risk assessment (QMRA) of pathogens in food safety is well established, but steps are being taken to expand this methodology to food spoilage. Parallels can be drawn between the steps involved in a QMRA for pathogens and its application to specific spoilage organisms (SSO). During hazard characterisation for pathogens, the appropriate dose–response model is used to link the hazard level to the health outcome by estimating the probability of illness, resulting from the ingestion of a certain dose of the hazard. The dose–response model, in the case of food spoilage, may be translated into a spoilage-response relationship linking the spoilage-level with the probability the consumer will discard the food and not consume it. Such models are developed with sensory testing, assessing consumers sensitivity to microbial spoilage quality defects and correlating them to the SSO concentration. Ignoring food spoilage before the stated expiration date can lead to the final health risk being overestimated, since cases in which the food item poses a real risk to the consumer but is not consumed due to perceived spoilage are not excluded. Plenty of risk assessments have been carried out for pathogens in different RTE foods. What is missing is the integration of the two approaches into a single model that can estimate the risk of illness, factoring in the variability of consumer responses to spoilage. The spoilage-response relationship was combined with a stochastic modelling approach for lactic acid bacteria (LAB) and *Listeria monocytogenes* growth, also taking into account microbial interaction between LAB and *L. monocytogenes* (Jameson effect) to increase accuracy. The comparison of results between the 'Baseline' and the 'Spoilage-informed' approach showed significant difference in listeriosis cases, both for consumers under and over 65 years old. These results may suggest, that the hypothesis about overestimation of listeriosis risk in case of not taking into account product spoilage is correct. The combined QMRA model developed in the present study can be a useful tool for risk management decisions in the meat industry.

KEYWORDS

deli meats, lactic acid bacteria, *Listeria monocytogenes*, listeriosis, predictive microbiology, QMRA, risk assessment, spoilage

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1 | INTRODUCTION

Due to a changing lifestyle, consumption of ready-to-eat (RTE) foods is constantly growing, because these foods are convenient, palatable and considered nutritious and safe. The main driver in choice of foods has always been taste, however convenience and health also factor in. RTE foods usually require no preparation before consuming, thus, it is highly important that these products are handled and stored properly, to avoid contamination and growth of pathogens, such as *Listeria monocytogenes* (Chambers et al., 2020; Smigic et al., 2023). *L. monocytogenes* is an intracellular foodborne pathogen that causes one of the most highly pathogenic foodborne zoonoses – listeriosis. Due to in part it's resistance to low temperatures, high salt concentration, low pH and low oxygen concentrations, it widely occurs in agricultural, aquaculture, food contact material and other niches in food processing plants (EFSA BIOHAZ Panel, 2018; Tirloni et al., 2024). Among healthy people, listeriosis is usually restricted to self-limiting febrile gastroenteritis, but seniors, young children and immuno-compromised people face a significantly higher risk of Listerial bacteremia, meningitis, meningoencephalitis and septicemia. Listeriosis also poses a serious risk for pregnant women, as it can lead to infection of the fetus, resulting in spontaneous abortion (Kurpas et al., 2018; Maćkiw et al., 2020).

The ability of *L. monocytogenes* to grow at low temperature deems it a serious hazard for RTE products with a longer shelf life (Maćkiw et al., 2020). According to several risk assessment studies of RTE foods, deli meats are of particular public health concern for listeriosis (EFSA BIOHAZ Panel, 2018; Pérez-Rodríguez et al., 2017). Cross-contamination of the final products generally occurs in processing plants or at the retail level (Kurpas et al., 2018). In the production process of RTE meat foods, it is crucial to use raw meat not contaminated with *L. monocytogenes*. In the scientific opinion by EFSA, storage time ought not to exceed 15 days for red and 3 days for poultry meat at appropriate temperature (EFSA, 2015). Despite this, contamination may also occur after processing and in the case of pre-packaged meats, this is the most likely scenario (Kurpas et al., 2018). In the European Union, *L. monocytogenes* continues to be a hazard with serious implications associated with high morbidity, hospitalisation and mortality rates (Pérez-Rodríguez et al., 2017). According to the latest EU Zoonoses Report, in 2022 there were 2738 confirmed listeriosis cases, 1330 hospitalisations and 286 deaths. In 2022 in the EU the overall occurrence of *L. monocytogenes* in RTE meat products was 2.1%. The overall trend for listeriosis did not reveal any significant changes between 2018 and 2022 (EFSA, 2022).

Food loss and waste pose a major problem in high-income countries (Durán-Sandoval et al., 2023). Only in the EU, over 58 million tonnes of food waste are generated yearly (EUROSTAT, 2023). A substantial contribution to this is food spoilage due to microbiological factors such as lactic acid bacteria (LAB), mainly when it occurs before the end of the stated shelf life (Karanth et al., 2023; Koutsoumanis et al., 2021; Tsaloumi & Koutsoumanis, 2024). Due to the rich nutrient composition, high-water activity and optimal pH, meat products are highly perishable (Karanth et al., 2023; Tsaloumi & Koutsoumanis, 2024). When concentrations of bacteria responsible for spoilage exceed 7–8 log CFU/g, spoilage becomes noticeable to consumers (Ghollasi-Mood et al., 2016; Tsaloumi et al., 2023). As shown in the study of Tsaloumi and Koutsoumanis (2024), for up to 4.5 days of storage of cooked ham products sliced at retail no spoilage events were observed but for 5 days of storage, the model predicted 1790 spoilage events for every 10,000 purchases. To obtain a robust study with regard to safety, the risk assessment of listeriosis and spoilage of cooked ham products should be combined, to avoid the possibility of overestimating the risk of listeriosis, when spoiled and uneaten products are not taken into account.

QMRA of pathogens in food safety is well established, but steps are being taken to expand this methodology to food spoilage (QMSRA) (Pouillot & Delignette-Muller, 2010). Similarities can be found between the steps involved in a QMRA for pathogen and its application to SSO. The most visible difference between QMRA and QMSRA can be observed during hazard characterisation. In the case of QMRA, the appropriate dose–response model is used to link the hazard level to the health outcome by estimating the probability of illness. On the other hand, in QMSRA, the dose–response model may be 'translated' into a spoilage-response relationship linking the spoilage level with the probability the consumer will discard the food and not consume it (Koutsoumanis et al., 2021). Dose–response models in spoilage risk assessment are developed with sensory testing, assessing consumers sensitivity to microbial spoilage quality defects and correlating them to the SSO concentration (Pérez-Rodríguez et al., 2017).

Based on the above, ignoring spoilage and product rejection in QMRA, may result in an overestimation of risk. The objective of the present study was the integration of two approaches into a single model that can estimate the risk of illness, factoring in the variability of consumer responses to spoilage.

2 | DESCRIPTION OF THE WORK PROGRAMME

2.1 | Aims

The EU-FORA fellowship, is a practical 'training-by-doing' programme that aims to improve knowledge and experience in food risk assessment among experts in Europe and to increase the EU's scientific assessment capacity and knowledge community (Bronzwaer et al., 2016). The undertaken programme guided the fellow in the process of conducting a RA and incorporating the variability of consumer spoilage perception into a QMRA model to develop an applied combined model for the pathogen *L. monocytogenes* and the SSO LAB in pre-packaged RTE deli meats.

2.2 | Activities/methods

2.2.1 | Sampling

In order to conduct a survey on the initial LAB concentration at the time of purchase and the physicochemical characteristics of cooked pre-packaged ham affecting LAB growth (pH, a_w, nitrite concentration) 30 packages of pre-packaged RTE cooked ham were purchased randomly from retail stores in central Poland. After purchase, products were transported to the laboratory under refrigerated conditions and were immediately subjected to microbiological and physicochemical analysis.

2.2.2 | Microbiological analysis

In order to estimate the initial concentration of LAB, microbiological analysis was performed according to the PN ISO 15214:2002 (Polish Committee for Standarization 15214, 2002), with 10 g of product placed into a stomacher bag (BagFilter, Interscience, France). Then, 90 g of sterile buffered peptone water (Oxoid, Great Britain) were added and the contents were homogenised in a Stomacher mixer (BagMixer, Interscience, France) for 120 s at ambient temperature. Appropriate decimal dilutions were prepared, and inoculated onto plates with the pour-plate method with MRS (Oxoid, Great Britain) agar. The plates were then incubated aerobically at 30 ± 1°C for 72 h. Colonies of presumptive LAB were enumerated after incubation and the results were expressed as log CFU/g. In order to determine the initial concentration of *L. monocytogenes*, a detection analysis was performed according to the PN ISO method 11290-1:2017 (Polish Committee for Standarization 11290-1, 2017). Twenty-five grams of product was placed in a mixing bag (BagFilter, Interscience, France), 225 g of primary enrichment medium – half-Fraser broth (Oxoid, Great Britain) was added and the contents were homogenised in the same way as before. The primary enrichment was incubated at 30 ± 1°C for 25 ± 1 h. After incubation, 0.1 mL of the obtained sample was transferred to secondary enrichment medium – Fraser broth (Oxoid, Great Britain) and incubate for 24 ± 2 h at 37°C. The surface of selective plating medium – ALOA and OXFORD agar (Oxoid, Great Britain) was inoculated by means of a loop from both primary and secondary enrichments and incubated for 48 ± 2 h at 37°C.

2.2.3 | Physicochemical analysis

Approximately half a slice of ham was shredded into small pieces in order to measure water activity (Aqualab 4TE, METER Group, USA). For the pH measurement (Mettler Toledo, USA) 10 g of sample were homogenised with 10 g of distilled water and the measure was conducted according to the instrument instruction. The nitrite content was measured according to the reference method PN-EN 12014-4:2006 (Polish Committee for Standarization 12014-4, 2006).

2.2.4 | Consumer questionnaire

Since there was no data regarding pre-packaged cooked ham eating habits among polish people, a consumer questionnaire was conducted. Results from this survey were incorporated into the exposure assessment. The study included 184 inhabitants of three voivodeships of central Poland – lodzkie, mazowieckie and wielkopolskie. Questions were related to consumers personal characteristics, frequency of eating and portion size. The results of this survey are under consideration for publication for use in future risk assessments.

2.2.5 | Risk assessment

In order to conduct stochastic quantitative risk assessment of the listeriosis risk related to the consumption of pre-packaged sliced RTE meat products packaged in a modified atmosphere on plastic trays, the open-source programming language – R and the *mc2d* package were used (Maćkiw et al., 2020). In the study, Modular Process Risk Model methodology (MPRM) was used, covering daily assessments throughout domestic refrigerated storage (Nauta, 2007). Simultaneously, the growth of *L. monocytogenes* and LAB was modelled, assuming that if spoilage microbial growth is not taken into account, the risk will be overestimated due to product spoilage prior to the expiration date. To assess different scenarios, 6 modules were added, for 0, 1, 2, 3, 4 and 5 days of SSL.

2.2.5.1 | *Hazard characterisation*

For the hazard characterisation in QMRA for *L. monocytogenes*, the exponential dose–response (DR) model of WHO/FAO (2004) was used, as following:

$$P(\text{ill}; d, r) = 1 - \exp(-rd),$$

where *r* is a parameter of the dose–response equation which is translated as the probability for one cell to successfully initiate a response (illness) for a given portion and *d* ingested dose. For the hazard characterisation in QMSRA the spoilage-response relationship developed by Tsaloumi and Koutsoumanis (2024) was used. Authors conducted simultaneous microbiological and sensory analyses, to define the SL that is the concentration of the LAB at which consumer rejection occurs (Koutsoumanis et al., 2021).

2.2.5.2 | *Exposure assessment*

The concentration of LAB and *L. monocytogenes* in tested products at the time of opening the package were assessed based exclusively on the initial concentration of the package purchased randomly at different times at retail and predicted growth during storage at home. For the next days of domestic storage of an opened package of deli meat, a modular approach was applied. Growth under domestic storage was predicted using the FSSP model. The FSSP model is based on the Jameson effect approach, taking into account microbial interaction between LAB and *L. monocytogenes*, which is characterised by the inhibiting effect of the dominating microflora of different species in the product. This phenomenon determines that two coexisting population simultaneously stop growing when the maximum population concentration is reached (Bolívar et al., 2021; Mejholm et al., 2010; Mejlholm & Dalgaard, 2009). The cardinal parameter growth and growth boundary model were used to calculate the growth rates of *L. monocytogenes* and LAB. The differential form of the simple logistic model was used as the primary model, describing the microbial interactions between LAB and *L. monocytogenes* (Mejlholm & Dalgaard, 2007). The physicochemical characteristics of the tested products (a_w, pH, nitrite concentration) were described by probability distributions selected by fitting distributions to the data in @Risk (Lumivero, USA). Similarly, serving size, time of opening the package and the time of storage used to calculate the dose of the pathogen and dose of SSO were taken from conducted survey and described as probability distributions following fitting in @Risk.

2.2.5.3 | *Risk characterisation*

In QMRA for *L. monocytogenes*, the probability of illness per serving was calculated using Monte Carlo simulation in R script. A total of 10,000 iterations were run for the complete model. For LAB, the probability of spoilage was also calculated using Monte Carlo Simulation in R for the same number of iterations. As well as prevalence of *L. monocytogenes* (*P*), probability of spoilage (P_{spoilage}) was introduced into the calculation of the spoilage-informed probability of illness:

$$P_{\text{i positive}} \times P \times \left(1 - P_{\text{spoilage}}\right),$$

where $P_{\text{i positive}}$ is the probability of illness from the consumption of *Listeria* positive sliced deli meat.

In order to predict annual listeriosis cases, the total number of EO per year were estimated based on the conducted survey. The probability of illness (PI(approx)) for an individual exposed to D (Dose) cells was presumed to follow a normal distribution and was described in accordance with the central limit theorem as following:

$$\text{PI((approx)}: \text{Normal}),$$

where μ and σ are the average and standard deviation of the probability of illness. The amount of listeriosis cases per year is expected to follow a binomial process. Nevertheless, because of the high number of EO, a normal approximation of the binomial distribution (LC(approx)) was used following:

$$\text{LC(approx)} = \text{Normal}\left(\text{EO} \times \text{PI(approx)}; (\text{EO} \times \text{PI(approx)} \times (1 - \text{PI(approx)}))^{0.5}\right),$$

where PI(approx) is the normal approximation of the probability of illness (PI) for an individual exposed to D cells (Dose) (Vose, 2008).

3 | RESULTS

The parameters of the developed risk assessment model are provided in Table A1 in Appendix A (Tables A2 and A3). Due to no samples testing positive, the *L. monocytogenes* prevalence of the products was described based on the report of EFSA (EFSA BIOHAZ Panel, 2018). The *Listeria* prevalence in pre-packaged deli meats (*P*) was described as a Beta distribution (Chambers et al., 2020). Similarly, the initial concentration of *L. monocytogenes* was described based upon the study of the EFSA BIOHAZ Panel (2018) as a Beta general. Initial contamination of LAB was based on experimental data. The tested products were characterised by relatively high concentrations of LAB, but in 8 out of 30 packages the concentration was below the detection limit (< 1 log CFU/g). Based on this, it was decided to base the initial concentration of LAB on certain assumptions: (i) instead of results below detection limit, the worst-case scenario of 1 log CFU/g was used; (ii) for the remaining 22 results, a normal distribution fitted to the data was used. The above parameters were used to characterise the prevalence of *L. monocytogenes* and initial concentration of *L. monocytogenes* and LAB, purchased at different time from a retail, based on the following assumptions: (i) the *L. monocytogenes* prevalence in the study from EFSA is representative

of Poland, (ii) initial concentration of *L. monocytogenes* from the EFSA BIOHAZ opinion is representative of Poland, (iii) the assumed minimum value for the initial concentration of LAB was appropriate.

The growth of LAB and *L. monocytogenes* in the analysed products during domestic storage in modified and ambient atmosphere was predicted using the FSSP growth model, validated by Tsaloumi and Koutsoumanis (2024) on sliced RTE deli meats. The maximum density of LAB and *L. monocytogenes* population are default values in the FSSP software, set to 8.5 log CFU/g. The variability of product characteristics was described with the use of probability distributions fitted to the data estimated in the present study. A logistic distribution was used for describing pH, water activity was described by a Pert distribution and nitrites were expressed as a Beta general distribution (Stefanou, 2022). A normal distribution was used to describe temperature in polish domestic refrigerators, based on the study of Stefanou et al. (2023). The minimum storage time (time of opening the package and first consumption) in domestic refrigerators was described with a cumulative distribution, based on data collected during a survey among polish consumers. According to data obtained in the previously mentioned survey and information on SSL provided on the packaging labelling of polish deli meats, it was decided to set maximum storage time as different scenarios, every 24 h, up to 5 days after opening.

Based on the present study, the serving size per eating occasion was estimated. A pert distribution was used to describe the serving sizes, assuming that there are no differences in serving sizes between consumers under and over 65 years of age. Serving size was set to multiples of individual slices of 10 g, which was found to be the average slice weight in the lab. The initial package net weight was set to 100 g and the daily consumption events were simulated up to the point the product is fully consumed or the final SSL date is reached. In order to calculate the number of cases, the annual EO of RTE deli meats was needed. Based on the conducted survey, consumers of RTE meats were extrapolated to the total population of Poland, according to the latest Demographic Yearbook of Poland (Główny Urząd Statystyczny/Statistics Poland, 2023). In this extrapolation, with 41 out of 184 (22.28%) participants not consuming RTE deli meat products were included. Due to the different susceptibility to listeriosis among people under and over 65 years of age (different r parameter value in the dose–response model) it was decided to calculate the total number of consumers separately for these two groups. The obtained results were multiplied by the number of eating occasions per year per consumer according to the frequency of consumption based on the conducted survey with the assumption that there is no difference in the percentage of consumers not consuming RTE deli meat between consumers of the two age groups. In order to obtain the probability of spoilage, the concentration values of LAB were entered into the spoilage-response relationship equation.

The probability of illness per serving for two age groups and each SSL scenario was estimated using Monte Carlo Simulation. Two approaches of calculating the probability of illness were used. The 'Baseline' approach did not take into account the probability of spoilage resulting from the simultaneous growth of LAB in the products, something we hypothesised would result in the overestimation of the risk, whilst the 'Spoilage-informed' approach took into account the probability of spoilage in calculating the probability of illness. The predicted 95th percentile of the listeriosis cases for consumption at the time of opening the package, was 41 cases for the 'Baseline' approach and 4 cases for the 'Spoilage-informed' approach for consumers < 65 years old. In case of consumers > 65 years old, the 95th percentile of cases at the time of opening was predicted to be 371 and 29 for the 'Baseline' and 'Spoilage-informed' approaches respectively. During the storage of an open deli meat package in the domestic refrigerator, the 'Spoilage-informed' approach shows a slight increase in the expected number of cases for increasing SSL scenarios from 0 to 5 days after opening, compared to the sharper increase noted in the 'Baseline' approach. On the fifth day of storage, for consumers < 65 years old, the 95th percentile of predicted cases was 91 and 7 for the 'Baseline' and 'Spoilage-informed' approaches respectively. For consumers > 65 years old, in the same circumstances, the number of cases was 870 for the 'Baseline' and 50 for the 'Spoilage-informed' approach. The highest number of cases (99th percentile) for the 'Baseline' approach among consumers < 65 years old, in subsequent SSL length scenarios was 45, 59, 72, 82, 100 and 100 for 0, 1, 2, 3, 4 and 5 days of SSL respectively. In the same situation, according to the 'Spoilage-informed' approach, the number of predicted cases was 5, 7, 9, 8, 8 and 8. On the other hand, 99th percentile of the predicted cases among consumers > 65 years old, was 384, 518, 647, 745, 890, 890 at 'Baseline' approach and 32, 47, 61, 56, 55, 55 at 'Spoilage-informed' approach for the same time condition as before. Among both consumer groups, the differences in the number of cases between the two approaches are of considerable magnitude.

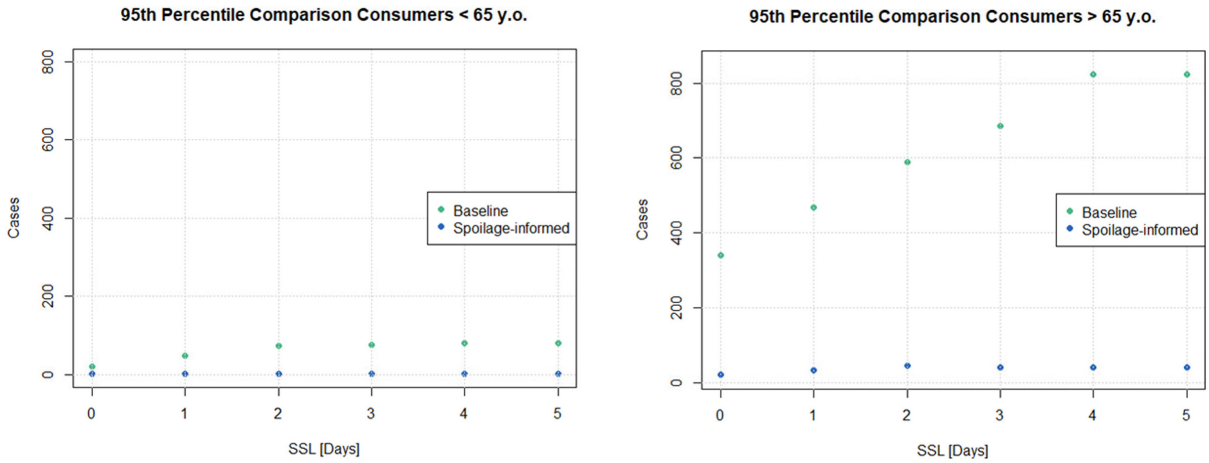

FIGURE 1 The effect of secondary shelf life (SSL): Comparison of the predicted 95th percentile of listeriosis cases between the 'Baseline' and 'Spoilage-informed' approach and between consumers < 65 years old (left picture) and > 65 years old (right picture) for different SSL scenarios. The *y*-axis shows cases and the x-axis shows the assigned SSL: SSL 0 = time of opening the package, SSL 1 = up to 1 day after opening, SSL 2 = up to 2 days of opening, SSL 3 = up to 3 days after opening, SSL 4 = up to 4 days after opening, SSL 5 = up to 5 days after opening.

Figure 1 presents the comparison of predicted 95th percentile of listeriosis cases between the 'Baseline' and 'Spoilage-informed' approach and between consumers < 65 years old and > 65 years old for six SSL scenarios (time of opening the package and up to 5 days of storage of the opened package in the fridge). A clear upward trend was observed in the case of the 'Baseline' approach, while the 'Spoilage-informed' approach was characterised by quite a stable trend.

4 | CONCLUSION

The fellowship aimed to acquaint the fellow with QMRA and QMRSA. The programme covered each step of the risk assessment process expertly guiding the fellow through tutoring and allowing the fellow to understand the entire methodology and build their own combined RA model in R. The developed spoilage-informed QMRA model is a first approach to assess the risk of listeriosis taking into account also the possibility of product spoilage before the stated expiration date. Furthermore, this study is the first study to assess the risk of listeriosis associated with the consumption of pre-packaged RTE deli meats available on the Polish market. Comparing results between the 'Baseline' and 'Spoilage-informed' approaches a significant reduction in expected listeriosis cases is evident, both for consumers under and over 65 years old. This may suggest that the listeriosis risk can be overestimated when excluding product spoilage. Moreover, due to the lack of data on the consumption habits for RTE deli meat among Poles, a consumption survey was conducted the results of which, once published, can be used in the future risk assessments.

5 | ADDITIONAL ACTIVITIES

Throughout the fellowship opportunities for further learning were provided by lectures and workshops related to food safety and RA. Tutoring on topics of EU Food Law, Microbial spoilage, Predictive Microbiology and a 2-week course in R programming and RA in R was provided to the fellow. A social programme was also organised to showcase local Greek food producers. Additionally, in September the fellow will present their work at the 10th International Conference on the Quality and Safety in Food Production Chain in Wroclaw, Poland. In November, the fellow is scheduled to give an oral presentation to IBPRS institute staff to present the progress and results of the EU-FORA fellowship programme. The work carried out in the project is also to be presented at the 38th EFFoST International Conference 2024 in Bruges, Belgium.

ABBREVIATIONS

CFU	colony forming units
EO	eating occasions
EU-FORA	European Union Food Risk Assessment
FAO	Food and Agriculture Organization
FSSP	Food Safety and Spoilage Predictor
LAB	lactic acid bacteria
MRS	de Man, Rogosa and Sharpe agar
QMRA	quantitative microbiological risk assessment
QMSRA	quantitative microbiological spoilage risk assessment
RA	risk assessment
RTE	ready-to-eat

SL spoilage-level
SSL secondary shelf life
SSO specific spoilage organism
WHO World Health Organization

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APPENDIX A

TABLE A1 Parameters of the risk assessment model.

Parameters	Units	Description	Calculations/distributions	Data source
P	–	Prevalence of *Listeria monocytogenes*	Beta distribution with parameters $a1 = 937.33$ $(s+1)$ and $a2 = 39774.67$ $(n-s+1)$	EFSA (2022)
C_{LAB}	CFU/g	Initial concentration of LAB	If (Binomial distribution (1, 0.267) = 0; 1; Normal distribution (4.19, 2.45))	Data from the present study
C_{LM}	CFU/g	Initial concentration of *L. monocytogenes*	Beta general distribution with parameters $\alpha1 = 0.502$, $\alpha2 = 2908$, min = −1.69, max = 6	EFSA BIOHAZ Panel (2018)
G_{LAB}	CFU/g	Growth of LAB in sliced pre-packaged deli meat product during domestic storage	Calculated with the FSSP model based on pH, a_w and nitrite of product and temperature of domestic refrigerators	Dalgaard (2014)
G_{LM}	CFU/g	Growth of *L. monocytogenes* in sliced pre-packaged deli meat product during domestic storage	Calculated with the FSSP model based on pH, a_w and nitrite of product and temperature of domestic refrigerators	Dalgaard (2014)
pH	–	pH of the product	Logistic distribution with parameters $\alpha = 6.15$, $\beta = 0.12$	Data from the present study
a_w	–	a_w of the product	Pert distribution with parameters min = 0.943, mode = 0.981, max = 0.982	Data from the present study
Nitrite	ppm	Nitrites of the product	Beta general distribution with parameters $\alpha1 = 0.838$, $\alpha2 = 8.483$, min = 0, max = 150	Data from the present study
T	°C	Temperature of domestic refrigerators in Poland	Normal distribution with parameters mean = 5.1, SD = 2.8, truncation(min = −1.2, max = 15.2)	Stefanou et al. (2023)
t_{min}	h	Minimum storage time of sliced pre-packaged deli meat at domestic refrigerators	Cumulative distribution	Data from the present study[TBP]
t_{max}	h	Maximum storage time of sliced pre-packaged deli meat at domestic refrigerators	120	Data from the present study[TBP]
Cc	CFU/g	Concentration consumed	$Cc = C_0 \times S$	–
S	g	Serving size	Pert distribution with parameters min = 10, mode = 20, max = 80	Data from the present study[TBP]
d	CFU/g	Dose	$d = Cc \times S$	–
$r_{<65}$	–	Dose–response model r parameter for consumers < 65 years old	2.37×10^{-14}	WHO/FAO (2004)
$r_{>65}$	–	Dose–response model r parameter for consumers > 65 years old	1.06×10^{-12}	WHO/FAO (2004)
$P_{i\ positive}$	–	Probability of illness from the consumption of a positive sliced deli meat portion	$P(\text{ill}; d, r) = 1 - \exp(-rd)$	WHO/FAO (2004)
$P_{spoilage}$	–	Probability of spoilage	Beta general distribution with parameters $\alpha1 = 0.92923$, $\alpha2 = 2.1897$, min = 7.0257, max = 8.1181	Tsaloumi and Koutsoumanis (2024)
$P_{i\_baseline}$	–	Probability of illness from the consumption of any sliced product	$P_{i\ positive} \times P$	–
$P_{i\_spoilage\_informed}$	–	Probability of illness from the consumption of any sliced non spoiled product	$P_{i\ positive} \times P \times (1 - P_{spoilage})$	–
$EO_{<65}$	–	Eating Occasions per year for consumer < 65 years old	9.7E+09	Data from the present study[TBP]
$EO_{>65}$	–	Eating Occasions per year for consumer > 65 years old	2.3E+09	Data from the present study[TBP]

TABLE A1 (Continued)

Parameters	Units	Description	Calculations/distributions	Data source
PI(approx)$_{<65}$	–	Probability of illness for consumers < 65 years old exposed to *d* (Dose)	Normal(μ; $\sigma/\sqrt{EO}$ < 65) where μ and σ are the average and SD of PI(approx)$_{<65}$	Vose (2008)
PI(approx)$_{>65}$	–	Probability of illness for consumers > 65 years old exposed to *d* (Dose)	Normal(μ; $\sigma/\sqrt{EO}$ > 65) where μ and σ are the average and SD of PI(approx)$_{>65}$	Vose (2008)
LC(approx)$_{<65}$	–	Annual number of listeriosis cases for consumers < 65 years old	Normal distribution with average: EO × PI(approx)$_{<65}$ and SD: $(EO \times PI(approx)_{<65} \times (1 - PI(approx)_{<65}))^{0.5}$	Vose (2008)
LC(approx)$_{<65}$	–	Annual number of listeriosis cases for consumers > 65 years old	Normal distribution with average: EO × PI(approx)$_{>65}$ and SD: $(EO \times PI(approx)_{>65} \times (1 - PI(approx)_{>65}))^{0.5}$	Vose (2008)

Abbreviation: TBP, to be published.

TABLE A2 Predicted yearly listeriosis cases among consumer < 65 years old in Poland linked with the consumption of pre-packaged RTE meat products presented in two approaches.

	SSL 0		SSL 1		SSL 2		SSL 3		SSL 4		SSL 5	
	B	S-I	*B*	S-I	*B*	S-I	*B*	S-I	*B*	S-I	*B*	S-I
Mean	32	2	43	3	55	4	64	4	76	4	76	4
SD	6	1	7	2	7	2	8	2	9	2	9	2
Min	5	5	17	0	27	0	35	0	43	3	44	7
Max	52	7	69	10	85	12	97	11	106	11	110	12
95th percentile	41	4	54	6	67	8	76	7	91	7	91	7
99th percentile	45	5	59	7	72	9	82	8	100	8	100	8

Abbeviations: *B*, Baseline approach; RTE, ready-to-eat; S-I, Spoilage-informed approach; SSL, Secondary shelf life.

TABLE A3 Predicted yearly listeriosis cases among consumer > 65 years old in Poland linked with the consumption of pre-packaged RTE meat products presented in two approaches.

	SSL 0		SSL 1		SSL 2		SSL 3		SSL 4		SSL 5	
	B	S-I	*B*	S-I	*B*	S-I	*B*	S-I	*B*	S-I	*B*	S-I
Mean	341	21	467	33	589	45	685	41	824	40	822	40
SD	18	5	22	6	24	7	26	6	29	6	29	6
Min	255	3	381	13	500	19	591	17	713	16	715	14
Max	406	38	551	56	689	69	797	65	922	63	932	66
95th percentile	371	29	502	42	630	57	730	52	871	50	870	50
99th percentile	384	32	518	47	647	61	745	56	890	55	890	55

Abbeviations: *B*, Baseline approach; RTE, ready-to-eat; S-I, Spoilage-informed approach; SSL, Secondary shelf life.