Approved: 23 September 2024

DOI: 10.2903/j.efsa.2024.e221113

EU-FORA SERIES 7

Q4MRATools: Quantitative tools to microbial risk assessment

Olga María Bonilla Luque[1] | **Antonio Valero**[1] | **Arícia Possas**[1] | **Styliani Roufou**[2] | **Jefferson de Oliveira Mallia**[3] | **Vasilis Valdramidis**[2]

[1]Department of Food Science and Technology, UIC Zoonosis y Enfermedades Emergentes (ENZOEM), CeiA3, Universidad de Córdoba, Córdoba, Spain

[2]Laboratory of Food Chemistry, Department of Chemistry, Zografou, National and Kapodistrian University of Athens, Athens, Greece

[3]Center for Molecular Medicine and Biobanking, University of Malta, Msida, Malta

Correspondence: eu-fora@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

In the context of the European Food Risk Assessment (EU-FORA) fellowship programme, the project 'Q4MRATools: Quantitative Tools to Microbial Risk Assessment' focused on training in predictive microbiology, experimental design and the use of advanced software tools like R, MATLAB, @Risk, DMFit and GInaFiT. The primary objective of this programme was to equip the fellow with foundational knowledge in quantitative microbial risk assessments (QMRA), thereby contributing to the development of more effective and accurate food safety risk assessments. This initiative was part of a broader effort to address the evolving challenges in food safety by enhancing collaborative actions and developing robust food safety systems. The fellow engaged in various risk assessment tasks, acquiring fundamental knowledge in predictive microbiology, particularly different modelling strategies for growth and inactivation models, as well as understanding the nuances of microbiological behaviour under different conditions and food matrixes environments. The training emphasised the importance of experimental design and the application of software tools essential for conducting QMRA. Secondary activities were also included to broaden the fellow's competencies, expanding their expertise beyond qualitative methods.

KEYWORDS

exposure assessment, fermented products, food safety, *Listeria monocytogenes*, pH, predictive microbiology, risk analysis

CONTENTS

Abstract 1
Summary 3
1. Introduction 4
    1.1. Background and Terms of Reference as provided by the requestor 4
2. Data and Methodology 4
    2.1. Data 4
    2.2. Methodology 5
        2.2.1. Computational activities 5
        2.2.2. Secondary scientific outcomes 6
3. Assessment 6
4. Conclusion 7
5. Recommendations 8
Abbreviations 8
Acknowledgements 8
Conflict of interest 8
Copyright for non-EFSA content 8
References 8

SUMMARY

The final report of the EU-FORA fellowship programme, titled 'Q4MRATools: Quantitative Tools to Microbial Risk Assessment,' outlines the activities undertaken by the fellow during the programme's implementation. This programme has been hosted by the Department of Chemistry, National and Kapodistrian University of Athens (NKUA) under the supervision of Prof. Vasilis P. Valdramidis, awarded by EFSA to the University of Córdoba (UCO) in Spain as sending institution. While the report includes only a portion of the data obtained, as the research is ongoing, a separate scientific publication will be developed to present the full outcomes.

The research concentrated on two main areas: (i) the assessment of the inhibitory activity of acids on various food-borne microorganisms and, (ii) the development of a quantitative microbial risk assessment (QMRA) of *Listeria monocytogenes* in artisanal Spanish fermented sausages. Preliminary results discussed the impact of pH on microbial growth, exploring how pH and temperature are influencing food safety. For the QMRA model, the fellow designed and implemented various variables to assess the risks associated with *L. monocytogenes* in artisanal sausages.

During the fellowship, the trainee has been working on the design and generation of informative experimental data to build the QMRA model structure, to define the input variables and to capture the variability and uncertainty sources describing the risk of *L. monocytogenes* in artisanal fermented sausages. Additionally, the fellowship aimed to familiarise the trainee with the acquisition of skills in software and model simulation tools that can be used to develop decision-making and QMRA in food microbiology. Methodologies employed in this research included extensive data collection, development of predictive models and simulation of food processing and preservation scenarios. The fellow utilised software tools like R Studio, @Risk and MicroHibro to implement predictive models, aiding in the understanding of the interaction between pH, temperature and microbial growth/inactivation. These models are crucial for enhancing food safety by predicting and controlling microbial contamination, particularly in RTE products.

The report highlights the importance of understanding the intricate interactions between pH, temperature and microbial growth to improve food safety standards. The fellow's contributions to the project align with the broader goal of refining risk assessment models and ensuring that food industry practices comply with food safety criteria. The EU-FORA fellowship played a pivotal role in equipping the trainee with the skills needed to critically review data and existing microbial risk assessments of *L. monocytogenes* and its implications for public health in the EU.

1 | INTRODUCTION

The ongoing challenge of feeding a growing population, while ensuring the freshness and safety of food by inhibiting microbial growth, continues to require thorough research from food scientists. Particularly, preventing and controlling microbial contamination in ready-to-eat (RTE) products is an essential task to guarantee food safety (Mataragas et al., 2010).

The consumption of RTE foods is steadily rising in high-income nations (Monteiro et al., 2013). These foods are often produced using a mixture of various raw materials under various processing conditions, whether in domestic, artisanal or industrial facilities, frequently involving extensive food handling, specialised manufacturing and preservation methods (Holck et al., 2017). Consequently, incidents of food contamination may occur more often in this category than in others, potentially originating from the use of contaminated raw materials, cross-contamination events, processing environments and equipment, or food handling practices (Bonilla-Luque et al., 2023). This scenario became particularly complex if considering the emerging new consumer trends and introduction of novel foods and changes in food products' composition.

To identify practices increasing the risk of food contamination and to support the compliance with current microbiological criteria, it is crucial to evaluate and understand the influence of extrinsic and intrinsic factors that affect microbial behaviour in RTE foods (Possas et al., 2021). For instance, the application of starter cultures (lactic-acid bacteria, LAB) or natural food preservatives as an alternative to antibacterial and antifungals, requires better understanding of the effect of organic acids which inhibit growth of spoilage organisms. In this sense, scientific knowledge across all these fields requires a deeper understanding of the impact of low pH, particularly when considered together with other factors and variables present in food (i.e. temperature), and how they are collectively affecting microbial behaviour.

Predictive microbiology tools help in assessing the responses of food-borne pathogens in various food matrices. Mathematical models have been developed to describe the effects of numerous factors on microbial growth in different food systems (Pérez-Rodríguez & Valero, 2013). These models, supported by reliable data, assist food operators in ensuring compliance with safety microbiological criteria. Predictive tools also bolster risk mitigation strategies, refine Hazard Analysis and Critical Control Points (HACCP) protocols, and support advanced methodologies like Quantitative Microbial Risk Assessments (QMRA). In this regard, QMRA is gaining recognition as a valuable tool for integrating necessary information to evaluate and prevent potential risks associated with food-borne pathogens.

The European Food Risk Assessment Fellowship (EU-FORA) is a hands-on 'learning by doing' programme designed to expand the pool of food safety risk assessment experts in Europe and to enhance Member States' participation in risk assessment activities (Bronzwaer et al., 2016). Through participation in the 7th EU-FORA cycle (2023–2024), a fellow from the Dpt. Food Science and Technology at the University of Córdoba (UCO) has been involved in the fellowship programme 'Q4MRATools: Quantitative Tools for Microbial Risk Assessment'. This fellowship programme, implemented by the Department of Food Chemistry at the National and Kapodistrian University of Athens (NKUA), Greece, under the supervision of Prof. Vasilis Valdramidis and coordinated by Antonio Valero and Arícia Possas from the sending institution, focuses on two key areas related to low-acid food products: (i) understanding the role of factors such as pH and temperature in pH-modified products (e.g. beverages, sauces and milk) on food-borne pathogens behaviour and, (ii) designing a QMRA for *L. monocytogenes* in artisanal Spanish fermented sausages, which are often associated with food safety alerts (AESAN, 2021, 2023; BOJA, 2024). The outcomes of this work provide a foundation for risk assessment calculations under various food processing and preservation scenarios.

1.1 | Background and Terms of Reference as provided by the requestor

The research groups and supervisors from the Food Chemistry at NKUA and Food Science and Technology Departments at UCO possess extensive expertise in developing predictive microbiology models and software tools for QMRA. This expertise enabled them to offer the EU-FORA fellow an opportunity to gain knowledge in the methodologies, terminologies, and practical skills commonly employed in QMRA. Since the fellow's background is based on veterinary and public health, the guidance and involvement of the supervisors during this fellowship facilitated the fellow to gain hands-on experience in modern methodologies and software tools for constructing QMRA models. This challenge was greatly facilitated by the fact that all the food matrices and topics were selected and carefully design in the project to align with the framework of the fellow's doctoral thesis (microbial safety and quality of mediterranean artisanal fermented foods).

2 | DATA AND METHODOLOGY

2.1 | Data

The EU-FORA programme provided advanced training in the application of mathematical and statistical methods to analyse data related to the microbial behaviour of pathogens in low pH food products across various categories. Initially, the fellow engaged in the working activities at the hosting site through the access to an extensive database provided by an ongoing initiative within the EuroMicropH COST Action. The input data, sourced from Combase® and Symprevius® with the special collaboration and co-work with the founders, included microbiological inactivation data obtained through challenge testing. This dataset contained information such as the study source, number of replicates, species of microorganisms tested, food matrices (including beverages, milk and sauces), pH, temperature, water activity (a_w) and microbial

concentrations (log cfu/g or mL) across experimental time points. The fellow was instructed on data extraction procedures and actively participated in applying exclusion criteria for studies and data in subsequent analyses. In a separate study, the fellow also worked with data obtained experimentally in artisanal Spanish fermented sausages. These additional data were collected from planned experiments conducted at the fellow's institution during the remote stage of the fellowship. This dataset focused on the growth behaviour of *L. monocytogenes* in fermented sausages during their ripening period, replicating conditions found in Spanish artisanal industries and assessing the impact of incorporating starter cultures, the behaviour of indigenous LAB and pH changes.

2.2 | Methodology

The primary focus of the activities involved training participants in the development and application of predictive models designed to assess microbial responses related to survival, growth and the likelihood of microbial recovery. The objectives of the programme were threefold: (i) to enhance the trainee' abilities to design and create meaningful experimental data, (ii) to develop an understanding of model structure development and selection to quantitatively describe microbiological phenomena while accurately measuring sources of stochasticity and (iii) to familiarise trainee with optimisation software and modelling simulations applicable for decision-making and QMRA in the field of food microbiology.

During the 1-year fellowship, the participant gained valuable insights into QMRA methods, both through remote learning and on-site experiences. Initial training was conducted online, with structured follow-up sessions with supervisors. Key training activities included mastering advanced statistical techniques and developing dedicated predictive models. The fellow also received guided learning on various specialised topics, such as: (1) utilising databases (EFSA, FAO, ComBase) for hazard identification; (2) formulating models based on published findings from online databases; (3) employing statistical principles in experimental design to analyse microbial growth and inactivation kinetics for constructing QMRA models; (4) acquiring a statistical programming language; and (5) interpreting the outcomes from analyses and QMRA simulations. This comprehensive approach provided the fellow with a solid theoretical foundation necessary for conducting QMRA, supplementing her prior education and training, including the 3-week introductory programme in microbiological and chemical risk assessment offered by EFSA.

Following this, the fellow has completed a 3-month in-person training at the Dpt. Food Chemistry at NKUA. This period involved practical experience and hands-on training in all required steps and tools for analysing large datasets and integrating this information into an exposure assessment framework for food-borne pathogens specifically in artisanal RTE foods, allowing her to perform her own QMRA. Probabilistic models were constructed using software tools like R Studio, @ Risk and MicroHibro, to represent various food supply chains within the QMRA framework. The training covered all aspects of risk assessment, including hazard identification, exposure assessment, hazard characterisation and risk characterisation (Tiwari & Cummins, 2013). Notably, the main focus was on exposure assessment to evaluate the likelihood of a hazard being present in food and the quantity of that food consumed by the population, contributing to quantify individual and population risk (risk characterisation).

The specific activities were organised into four key tasks: (1) Training on data repositories, software applications and methodologies for gathering data to assess microbial exposure to *L. monocytogenes* in artisanal RTE foods. A thorough review was conducted to identify pertinent data sources, such as scientific publications, databases, literature and experimental studies. Data related to microbial hazards, food formulations, processing variables and consumption patterns were gathered and organised into predefined databases. (2) Conducting multivariate analysis of the microbial resistance in low-acid food products. During this stage, the fellow analysed data extracted from Combase® to determine inactivation kinetics parameters. The goal was to identify potential clusters linking inactivation parameters to pH levels, species of bacteria and other factors. Results from this task may be shared with the COST action (https://euromicroph.eu). (3) Developing predictive microbiological models for *L. monocytogenes* in artisanal RTE foods. This involved using data from challenge tests on *L. monocytogenes* in artisanal foods to fit various predictive models under both static and dynamic storage conditions, also assessing the impact of different biopreservation strategies. (4) Development of a stochastic model that includes critical stages of the production and distribution process for meat products, such as reception, mixing, ripening and storage. This task focused on constructing and integrating probabilistic models of *L. monocytogenes* into software tools for estimating the concentration and prevalence of the pathogens in artisanal RTE foods at the end of their distribution.

2.2.1 | Computational activities

Different data processing strategies were employed during the implementation of the work programme. The first approach involved utilising MATLAB and R software in parallel, and self-designed codes, while the process involved the familiarisation with various computational methods. The second approach involved MS Excel, enhanced with add-ins such as @ Risk, DMFit or GInaFiT. Each of them were applied according to the specific needs and objectives of the research.

2.2.2 | Secondary scientific outcomes

- Conference Participation: The fellow presented the work titled 'Assessing Antimicrobial Potential of Mediterranean Plant Extract (*Allium*, *Ocimum* and *Thymus* spp.) through a Meta-analytical Approach' at the 2024 European Symposium on Food Safety held from 30 April to 2 May, 2024, in Geneva, Switzerland. Additionally, the fellow attended the 28th International ICFMH Conference in Burgos, Spain, from 8 to 11 July, 2024, in which the fellow presented the oral communication titled 'Exploring the role of pH on the microbial responses of food-borne pathogens in food products stored under different temperatures'.
- Professional Visits: The fellow visited the headquarters of the Spanish Agency for Food Safety and Nutrition (AESAN) in Madrid and the National Centre for Food Laboratory in Majadahonda on 27 and 28 February, 2024. During this visit, the fellow benefited from presentations on AESAN's work in risk assessment, management and communication.
- EU-FORA Programme: The fellow participated in the EU-FORA programme, engaging in various modules offered in both online formats and in-person, in headquarters of the European Food Safety Authority (EFSA), in Parma, Italy.
- Knowledge Acquisition and Exchange: The fellow was involved in multiple activities at the hosting organisation, including attending presentations by master's and PhD students and working group meetings. The fellow also took part in workshops and summer schools focused on risk assessment, organised under the Innovative Training Networks (ITN) of the Marie Skłodowska-Curie Actions, specifically the TRANSIT project (H2020-MSCA-ITN-2020). Additionally, the fellow delivered a conference at the hosting site, discussing her professional trajectory, doctoral thesis plan, research results and ongoing work.
- Future Plans and Contributions: The fellow plans to submit one to two publications to international scientific journals to enhance visibility. A presentation is scheduled of the QMRA methodology for microbial risks in foods (lecture to undergraduate students at the University of NKUA).

3 | ASSESSMENT

Understanding the factors that influence the responses of food-borne pathogens is crucial for designing effective food safety measures. Hurdle technology, which considers multiple factors like pH and temperature to inhibit microbial growth, plays a key role in this process. In our first study, we analysed the impact of pH on pathogen responses in foods stored at varying temperatures. Data from 121 studies were modelled using GInaFiT to estimate Log-3-reduction (L3R) times. GInaFiT is a valuable tool enabling the testing of various microbial survival models (Geeraerd et al., 2000, 2005).

Subsequent analyses were conducted in parallel using R Studio and MATLAB. Due to the non-normal distribution of the data, all statistical analyses were performed using non-parametric methods. To detect preliminary differences influenced by pH, we categorised the data into two groups: food environments with pH below 4.5 and those with pH above 4.5. Statistically significant differences in L3R times were found between these two groups (Table 1). Additionally, correlations and significant relationships between various factors and variables derived from predictive models were identified. Heatmap analyses have shown that fewer results were observed for certain categories, particularly in higher pH foods, as only milk products were included in this group. The data revealed that microbial reduction times were longer in lower pH food products, which is consistent with previous studies where temperature and storage times were the affecting factors. In this sense, it is important to consider that factors in combination with pH, such as temperature, may influence these outcomes. Preliminary findings suggested that the temperatures used in the selected studies could be affecting microbial behaviour in low pH food products, possibly due to nutrient competition at higher temperatures (Possas et al., 2022). Further research is in process to better understand these relationships.

TABLE 1 Estimation of L3R for microbial inactivation of *Salmonella* spp., *Escherichia coli*, *Listeria innocua*, *Listeria monocytogenes* and *Campylobacter fetus* in beverages, sauces and milk food matrices.

	pH < 4.5			pH > 4.5		
	pH	Temp (°C)	L3R (min)	pH	Temp (°C)	L3R (min)
Minimum	3.20	4	24.74	6.49	1	17.77
Maximum	4.12	25	778.39	6.80	30	386.60
Median	3.77	8	243.13	6.60	14.04	119.76

Microbial resistance was ranked based on the L3R times. In the lower pH category, *Salmonella* spp., *Escherichia coli* and *L. monocytogenes* were the most studied pathogens, with *Salmonella enterica* identified as the most resistant (L3R of 495.91 min). In the milk product category, there were slight differences between the two most studied pathogens, i.e. *E. coli* and *Campylobacter jejuni*, although *E. coli* was the most resistant (L3R of 121.31 min). Moreover, within specific food products, significant differences were observed between microbial groups, such as between *E. coli* and *S. enterica* in beverages, and between *L. monocytogenes* and *E. coli* in sauces. Our findings aligned with previous in vitro assessments (Lin et al., 1995). However, our analyses studies conducted in various food matrices, allowed us to further capture different

microbial behaviours, such as variations between Gram-positive and Gram-negative bacteria, as well as interspecies and inter-strain variability.

In a second analysis, we focused on the design and construction of a QMRA specific to artisanal fermented sausages. The first stage involved modelling the growth of *L. monocytogenes* based on data obtained from laboratory experiments where artisanal fermented sausages were artificially contaminated (challenge test). This growth data was used to represent the behaviour of *L. monocytogenes* during the ripening stage of the sausage processing. Various cardinal models were selected, considering factors such as changing pH, reduction of a_w, growth of LAB and acid production. For instance, proposed a cardinal model that incorporates pH and undissociated lactic acid concentration, along with other factors like temperature, minimum growth temperature, pH and a_w.

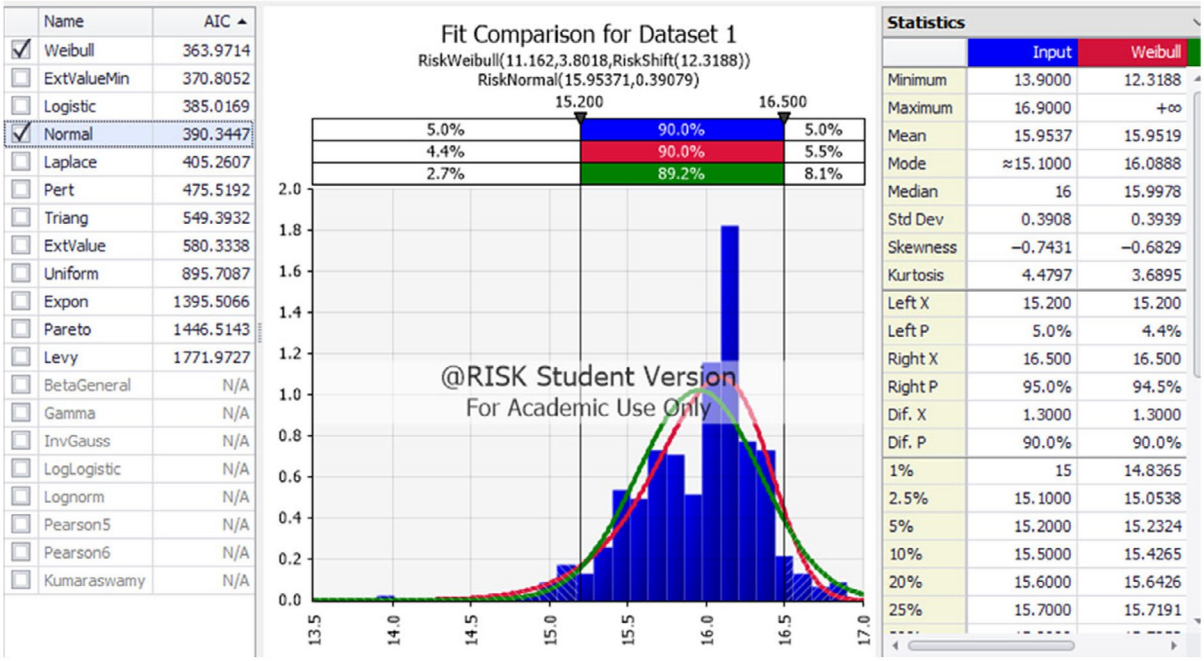

FIGURE 1 Caption of @Risk Microsoft Excel Add-in performing distribution fitting on ripening temperature data.

In the subsequent stages of storage, kinetic values were used to determine the final concentration of *L. monocytogenes*, including the probability of contamination, microbial concentration, weight of each sausage, microbial behaviour at reference temperature (5°C), minimum growth temperature and exponential growth rate. These values were obtained from both previous studies and our own data (Bonilla-Luque et al., 2024). The Rosso equation and Baranyi primary models were independently applied to estimate the concentration of *L. monocytogenes* at each storage stage (EFSA BIOHAZ Panel, 2018). Real-time temperature scenarios from artisanal production were incorporated using a deterministic approach combined with probabilistic distributions for each parameter. For instance, the most suitable statistical distribution was fitted using @Risk from a set of experimental temperatures (Figure 1) to describe variability in the microbial growth.

Furthermore, specific manufacturing stages, such as retail time – where 95% of the products are purchased by consumers – and the probability of temperature abuse during the consumer storage, were given in more detailed based on available information and considering the stage's impact on the final product safety. All the data and values were compiled in the same Excel file for calculations, organised by stages and facilitating the most practical visualisation.

Finally, the risk of listeriosis per serving and the adjustment of the product's shelf-life were determined. This assessment took into account an estimated serving size, which was divided into three age categories: above 65, between 18 and 64 and below 18 years old. The dose, expressed as log CFU, was also calculated for each age group.

4 | CONCLUSION

The primary objective of the work programme was to develop and apply predictive models to assess the impact of various factors on microbial inactivation and to estimate the public health risks associated with listeriosis from consuming artisanal Spanish fermented sausages. This programme provided the fellow with extensive expertise in conducting risk assessments for microbiological hazards. Throughout the programme, the fellow acquired the skills needed to design a risk assessment framework and construct a QMRA model by integrating information, experimental data and mathematical equations, while considering all relevant input parameters (such as temperature, time and microbial population) that influence the outcomes. A key aspect of this process was the fellow's familiarisation with pertinent online risk assessment tools, particularly the @Risk software package, which is recognised as a powerful tool for risk analysis. High-quality data, coupled

with effective risk communication, are crucial for conducting well-informed risk assessments and management, ensuring a robust risk analysis and achieving a high level of protection for human health and safety. The EU-FORA programme offered an invaluable opportunity to exchange ideas, methodologies and scientific insights, as well as an important step in establishing a professional network that will support future collaboration in risk assessment research.

5 | RECOMMENDATIONS

The fellowship programme significantly contributes to developing expertise in various stages of risk assessment. Additionally, a key strength of the programme is its focus on expanding the fellows' professional networks. The working groups established across different organisations are a vital resource provided by this programme, and can be effectively maintained by scheduling follow-up meetings in a manner that suits both departments.

ABBREVIATIONS

AESAN	Agencia española de Seguridad alimentaria y Nutrición
EUFORA	European Food Risk Assessment
FAO	Food and Agriculture Organization
HACCP	Hazard Analysis and Critical Control Points
L3R	Log-3-reduction
LAB	lactic acid bacteria
NKUA	National and Kapodistrian University of Athens
QMRA	quantitative microbial risk assessments
RTE	ready-to-eat
UCO	University of Córdoba

ACKNOWLEDGEMENTS

This report is funded by EFSA as part of the EU-FORA programme. Fellow wishes to thank the following for the support provided to this scientific output: Cristina Alonso Andicoberry, Francesca Manzoni, Christoph Unger and the fellows of this EU-FORA cohort.

REFERENCES

Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). (2021). Alerta por presencia de Listeria monocytogenes en embutidos procedentes de Cataluña (Ref. INF2021/0056). https://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/ampliacion/INF2021_0056.htm

Agencia Española de Seguridad Alimentaria y Nutrición (AESAN). (2023). Alerta por presencia de Listeria monocytogenes en productos cárnicos procedentes de España (Ref. ES2023/272). https://www.aesan.gob.es/AECOSAN/web/seguridad_alimentaria/ampliacion/2023_27.htm

Boletín Oficial de la Junta de Andalucía (BOJA). (2024). Actualización alerta alimentaria por presencia de listeria monocytogenes en producto cárnico (zurrapa) de andalucía. https://www.juntadeandalucia.es/organismos/saludyconsumo/areas/seguridad-alimentaria/alertas-alimentarias/paginas/alerta-listeria-zurrapa.html

Bonilla-Luque, O. M., Possas, A., Cabo, M. L., Rodríguez-López, P., & Valero, A. (2023). Tracking microbial quality, safety and environmental contamination sources in artisanal goat cheesemaking factories. *Food Microbiology*, *114*, 104301.

Bonilla-Luque, O. M., Valero, A., Tomasello, F., Cabo, M. L., Rodríguez-López, P., & Possas, A. (2024). Exploring microbial diversity during the artisanal Salchichon production: Food safety in the consumer spotlight. *LWT – Food Science and Technology*, *191*, 115550.

Bronzwaer, S., Le Gourierec, N., & Koulouris, S. (2016). Editorial: The European Food Risk Assessment Fellowship Programme (EU-FORA). *EFSA Journal*, *14*(11), 14111.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci, A., Allende, A., Bolton, D., Chemaly, M., Davies, R., Fernández Escámez, P. S., Girones, R., Herman, L., Koutsoumanis, K., Nørrung, B., Robertson, L., Ru, G., Sanaa, M., Simmons, M., Skandamis, P., Snary, E., Speybroeck, N., Ter Kuile, B., … Lindqvist, R. (2018). *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. *EFSA Journal*, *16*(1), 5134.

Geeraerd, A. H., Herremans, C. H., & Van Impe, J. F. (2000). Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, *59*, 185–209.

Geeraerd, A. H., Valdramidis, V. P., & Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, *102*, 95–105.

Holck, A., Axelsson, L., McLeod, A., Rode, T. M., & Heir, E. (2017). Health and safety considerations of fermented sausages. *Journal of Food Quality*, *2017*(1), 9753894.

Lin, J., Lee, I. S., Frey, J., Slonczewski, J. L., & Foster, J. W. (1995). Comparative analysis of extreme acid survival in *Salmonella typhimurium*, *Shigella flexneri*, and *Escherichia coli*. *Journal of Bacteriology*, *177*(14), 4097–4104.

Mataragas, M., Zwietering, M. H., Skandamis, P. N., & Drosinos, E. H. (2010). Quantitative microbiological risk assessment as a tool to obtain useful information for risk managers—Specific application to *Listeria monocytogenes* and ready-to-eat meat products. *International Journal of Food Microbiology*, *141*, S170–S179.

Monteiro, C. A., Moubarac, J. C., Cannon, G., Ng, S. W., & Popkin, B. (2013). Ultra-processed products are becoming dominant in the global food system. *Obesity Reviews*, *14*, 21–28.

Pérez-Rodríguez, F., & Valero, A. (2013). *Predictive microbiology in foods*. Springer.
Possas, A., Bonilla-Luque, O. M., & Valero, A. (2021). From cheese-making to consumption: Exploring the microbial safety of cheeses through predictive microbiology models. *Food*, *10*(2), 355.
Possas, A., Hernández, M., Esteban-Carbonero, Ó., Valero, A., & Rodríguez-Lázaro, D. (2022). *Listeria monocytogenes* survives better at lower storage temperatures in regular and low-salt soft and cured cheeses. *Food Microbiology*, *104*, 103979.
Tiwari, U., & Cummins, E. (2013). Risk analysis for a sustainable food chain. In B. K. Tiwari, T. Norton, & N. M. Holden (Eds.), *Sustainable food processing* (pp. 103–124). John Wiley & Sons.

How to cite this article: Luque, O. M. B., Valero, A., Possas, A., Roufou, S., de Oliveira Mallia, J., & Valdramidis, V. (2024). Q4MRATools: Quantitative tools to microbial risk assessment. *EFSA Journal*, *22*(S1), e221113. https://doi.org/10.2903/j.efsa.2024.e221113