

## Evaluation and prioritization of biological risk in Ready-To-Eat (RTE) meat products through mathematical modelling

## Evaluación y priorización del riesgo biológico en productos cárnicos listos para comer (LPC) a través de modelos matemáticos

L. Georgalis<sup>1\*</sup>, P.S. Fernández<sup>1</sup>, A.P. Garre<sup>1</sup>, A. Psaroulaki<sup>2</sup>

<sup>1</sup>Department of Agronomical Engineering, ETSIA. Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, 30203 Cartagena. Spain.

<sup>2</sup>Department of Clinical Microbiology and Microbial Pathogenesis, School of Medicine, University of Crete, Voutes-Staurakia, 71110 Heraklion, Crete. Greece.

\*leonidas.georgalis@upct.es

### **Abstract**

Modern food production has reduced the cost and increased the variety of food available, but this centralisation of the food supply presents an opportunity for foodborne pathogens and toxins to infect and poison large numbers of consumers. Modelling is a key tool to ensure the safety of food produced from raw material to the final consumer. The starting hypothesis is that it is possible to improve the safety of Ready To Eat (RTE) meat products by evaluation / prioritization of biological risks. Risk ranking of feed/food safety and nutritional related health risks is generally recognised as the starting point for risk-based priority setting and resource allocation, as it permits policymakers to allocate their resources on the most significant public health problem. The outcome of the thesis will be a robust tool for food safety management that can be implemented by all stakeholders, such as agencies related to consumer protection (EFSA, ECDC) and industry.

**Keywords:** food safety; risk assessment; foodborne disease.

### **Resumen**

La producción moderna de alimentos ha reducido el coste y ha aumentado la variedad de alimentos disponibles, pero esta centralización del suministro de alimentos presenta una oportunidad para que los patógenos y toxinas transmitidos por los alimentos infecten y envenenen a un gran número de consumidores. El modelado es una herramienta clave para garantizar la seguridad de los alimentos producidos a partir de materias primas para el consumidor final. La hipótesis inicial es que es posible mejorar la seguridad de los productos cárnicos listos para comer (LPC) mediante la evaluación/priorización de riesgos biológicos. La clasificación de riesgos de los riesgos para la salud de los alimentos y la seguridad alimentaria y nutricional se reconoce generalmente como el punto de partida para el establecimiento de prioridades basadas en el riesgo y la asignación de recursos, ya que permite a los encargados de formular políticas asignar sus recursos al problema de salud pública más importante. El resultado de esta tesis será una herramienta sólida de gestión de la inocuidad de los alimentos que puede ser implementada por todos los interesados, como las agencias de protección al consumidor (EFSA, ECDC) y la industria.

**Palabras clave:** seguridad alimentaria; evaluación de riesgos; enfermedad transmitida por alimentos.

## 1. INTRODUCTION

Mainly, two pathogens have been studied extensively the past decade, regarding their potential to produce foodborne illnesses. *Listeria monocytogenes*, the foodborne pathogen responsible for listeriosis, and *Escherichia coli*. Surprisingly, the number of reported confirmed listeriosis cases further increased in 2018, despite listeria rarely exceeding the EU food safety limit tested in ready-to-eat food. Therefore, further research is needed to fill-in the gaps of previous risk assessments for *L. monocytogenes* (1).

On the other hand, pathogens such as *Salmonella*, *Staphylococcus aureus* and *Campylobacter* need further investigation and justification about their role in RTE meat food products. The annual report on trends and sources of zoonoses, published by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) for the year 2018, showed that *Salmonella* is the most common cause of foodborne outbreaks in the European Union. In 2018, 91,857 confirmed cases of salmonellosis in humans were reported with an EU notification rate at 20.1 cases per 100,000 population (1). *S. aureus* is recognized as one of the major foodborne pathogens in fresh and ready-to-eat products and responsible for various infections around the world. Staphylococcal enterotoxins have been detected in many foodstuff causing staphylococcal food poisoning, toxic shock, and allergic and autoimmune reactions (2). Furthermore, Campylobacteriosis is the most commonly reported gastrointestinal disease in humans in the EU and has been so since 2005. In 2018, the number of confirmed cases of human campylobacteriosis was 246,571 corresponding to an EU notification rate of 64.1 per 100,000 population (1).

Because growth and survival under stressful conditions is the norm, the responses and survival of bacteria and other microbes during exposure to environmental stresses has become an important area of study in microbiology (3). Acclimation involves physiological, anatomical, or morphological adjustments within a single organism that improve performance or survival in response to environmental change. The extent of this acclimation is constrained by the genome of the individual (4). The ability to adapt rapidly to environmental change is essential for bacteria survival and virulence, and both stress responses and virulence genes are expressed in response to environmental signals (5).

Mathematical models used in predictive microbiology have a strong empirical nature, including some model parameters that must be estimated based on experimental data. The variability in the bacterial response of different strains, when exposed to similar conditions, are expressed by different values of the model parameters. As a result the microbial response is characterized as a serious issue for microbial risk assessment, due to the large number of different microbial strains that can potentially contaminate a good product before its use for prediction (6).

Therefore, risk assessment cannot be performed for every potential strain using the resources available nowadays. Instead, it is limited to some bacterial strains identified as the most resistant ones and/or the ones with the highest growth potential (7). This is exactly the gap that our study wants to fill in by testing different bacterial strains and mathematical models. Risk ranking of feed/food safety and nutritional related health risks is generally recognised as the starting point for risk-based priority setting and resource allocation, as it permits policymakers to allocate their resources on the most significant public health problem (8).

## 2. MATERIALS AND METHODS

### 2.1 Proposed objectives

In order to achieve the proposed goals, the following objectives are proposed:

A. Identification and enumeration of the most relevant pathogenic microorganisms (*Listeria monocytogenes*, *Campylobacter*, *Salmonella spp.*, *S. aureus*) in RTE cured meat of complex composition:

- A1. Determine the prevalence of the above microorganisms by food type, which have been related to national outbreaks.
- A2. Study the virulence of each pathogenic microorganism circulating on the market.
- A3. Characterise the nature and size of the microbial food safety risk.
- A4. Assess the effect of preservation treatments on the fate of pathogenic microorganisms in the final product.
- A5. Investigate the influence of physicochemical parameters (temperature, pH, water activity, NaCl) on contamination in RTE cured meat.

B. Establishment of safe conditions for moderate novel heat treatments:

- B1. Determination of the kinetics of inactivation and growth of pathogenic microorganisms in static conditions against non-thermal and moderate combined treatments.
- B2. Determination of the kinetics of inactivation and growth of pathogenic microorganisms under dynamic conditions.
- B3. Modelling the acclimation dynamics of the microorganisms.

C. Determination of sublethal damage for each of the selected microorganisms:

- C1. Growth in selective media of microorganisms exposed to damage-inducing treatments.
- C2. Pre-exposure to stress and growth conditions prior to thermal treatment could increase survival capability during processing.
- C3. Modelling of data obtained through frequency distributions and other appropriate statistical methods.

D. Analysis of the data obtained by establishing a risk prioritization or risk ranking of pathogenic microorganisms for each method evaluated in the food products:

- D1. Establishment of the most appropriate methodology in each pathogenic microorganism.

## 2.2 Description of samples

The samples to be used will be RTE meat products of complex composition related in outbreaks of food poisoning at Spanish/European level. Different technologies will be applied to design safe processes that allow the development of new food products.

## 2.3 Description of equipment

The unique instrumentation available for the development of the project consists of:

- Mastia thermoresistometer (9), available at the UPCT for the determination of microbial heat resistance in both isothermal and non-isothermal heating conditions. Working temperatures range from 20 to 150 °C and heating and cooling speeds of 40 °C / min.
- Tubular heat exchanger at pilot plant scale available at UPCT, with various sampling points along the heating section and flows around 1 L / min.
- Two bioscreen C devices for determination of bacterial growth with a high number of repetitions (up to 400 simultaneously) by optical density reading.
- Additionally, there are is a fully equipped microbiology laboratory.

## 3. RESULTS AND DISCUSSION

The scientific methodology associated with the evaluation of microbiological risks and the prioritization of microbiological risks will be used, obtaining the microbiological and technological data necessary for their correct integration into this methodology. Based on the data

and its correct statistical analysis, safe processing and storage conditions will be established, as well as the associated critical limits.

The outcome of the project will be a robust tool for food safety management that can be implemented by all stakeholders, such as agencies related to consumer protection (EFSA, ECDC) and industry.

#### 4. CONCLUSIONS

It has been shown that, although there are numerous scientific studies, the data available in certain areas related to emerging technologies and microorganisms are insufficient or have an inappropriate format for integration into current decision-making tools (evaluation of risks, establishing a risk prioritization or risk ranking, etc.), so it is necessary to identify these deficiencies and generate adequate data

#### 5. AGKNOWLEDGEMENS

Leonidas Georgalis is grateful to the MINECO for awarding him a pre-doctoral grant, through Project AGL2017-86840-C2-1-R. To EFSA for awarding him an EU FORA fellowship, 2019-2020.

#### 6. REFERENCES

1. The European Union One Health 2018 Zoonoses Report [Internet]. European Food Safety Authority. 2019 [cited 2020 Jan 24]. Available from: <https://www.efsa.europa.eu/en/efsajournal/pub/5926>
2. Gonano M, Hein I, Zangerl P, Rammelmayr A, Wagner M. Phenotypic and molecular characterization of *Staphylococcus aureus* strains of veterinary, dairy and human origin. *Epidemiol Infect.* 2009;137(5):688–99.
3. Spector MP, Kenyon WJ. Resistance and survival strategies of *Salmonella enterica* to environmental stresses. *Food Research International.* 2012;45(2):455–81.
4. Demmig-Adams B, Dumlao MR, Herzenach MK, Adams WW. Acclimation. In: Jørgensen SE, Fath BD, editors. *Encyclopedia of Ecology.* Oxford: Academic Press; 2008;15–23. <http://www.sciencedirect.com/science/article/pii/B978008045405400001X>
5. Shen S, Fang FC. Integrated Stress Responses in *Salmonella*. *Int J Food Microbiol.* 2012;152(3):75–81.
6. Hassani M, Cebrián G, Mañas P, Condón S, Pagán R. Induced thermotolerance under nonisothermal treatments of a heat sensitive and a resistant strain of *Staphylococcus aureus* in media of different pH. *Letters in Applied Microbiology.* 2006;43(6):619–24.
7. Garre A, Egea JA, Iguaz A, Palop A, Fernandez PS. Relevance of the Induced Stress Resistance When Identifying the Critical Microorganism for Microbial Risk Assessment. *Front Microbiol.* 2018;9:1663. [https://www.frontiersin.org/articles/10.3389/fmicb.2018.01663/full?utm\\_source=Email\\_to\\_authors&utm\\_medium=Email&utm\\_content=T1\\_11.5e1\\_author&utm\\_campaign=Email\\_publication&field=&journalName=Frontiers\\_in\\_Microbiology&id=386328](https://www.frontiersin.org/articles/10.3389/fmicb.2018.01663/full?utm_source=Email_to_authors&utm_medium=Email&utm_content=T1_11.5e1_author&utm_campaign=Email_publication&field=&journalName=Frontiers_in_Microbiology&id=386328)
8. Fels-Klerx HJ van der, Asselt ED van, Raley M, Poulsen M, Korsgaard H, Bredsdorff L, et al. Critical review of methodology and application of risk ranking for prioritisation of food and feed related issues, on the basis of the size of anticipated health impact. *EFSA Supporting Publications.* 2015;12(1):710E.
9. Conesa R, Andreu S, Fernández PS, Esnoz A, Palop A. Nonisothermal heat resistance determinations with the thermoresistometer Mastia. *Journal of Applied Microbiology.* 2009;107(2):506–13.