

# 13th ICPMF

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Predictive Modelling in Food



## ICPMF

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# Abstract Book

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## Part A – The old good times

### OP01

#### Growth kinetics of bacterial spore-formers isolated from plant-based ingredients: Consequences for food safety and quality

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Spores of *Bacillus* and *Clostridium* species are predominant in plant-based ingredients. The intrinsic characteristics of products derived thereof and the processing conditions may allow for growth of spore-formers during processing and shelf life. The aim of this study was to evaluate growth boundaries (temperature, pH, undissociated lactic acid [HLac]) of different spore-formers that were isolated from plant-based ingredients, benchmark to literature data and to verify cardinal model-based growth predictions in plant-based yogurt during fermentation.

For 17 plant-based ingredient isolates, comprising different *Clostridium* and *Bacillus* species, cardinal values for temperature, pH, and HLac were determined based on specific growth rates collected at 12-37 °C, pH 4.5-7.0, and 0-6 mM HLac at pH 5.5 in TSB, respectively. Additionally, cardinal values for the species were collected from literature to benchmark the experimental data. Growth in plant-based yogurt, containing faba protein, was assessed using one *Bacillus cereus* and one *Bacillus cytotoxicus* strain. A commercial starter culture was added to the faba yogurt base in different concentrations, resulting in different acidification rates. Growth of *B. cereus* and *B.*

*cytotoxicus*, pH of the yogurt and lactate formation were monitored over time for up to 24 hours.

Strains within the same species showed rather similar cardinal values for pH, HLac and temperature, which were also in the range reported in literature, allowing for Gamma concept-based growth predictions. Model predictions based on the Gamma concept for temperature, lactate and pH, indicated that the fermentation rate is very important to inhibit growth of *B. cereus* and *B. cytotoxicus* during fermentation of plant-based yogurt. This was confirmed by experimental data. Also, a clear matrix effect of the faba yogurt was observed.

In conclusion, this study shows growth of *Bacillus* and *Clostridium* species isolated from plant-based ingredients at temperatures and at pH and lactate concentrations that are relevant to plant-based yogurt and other plant-based products. Model predictions, based on cardinal values from plant-based isolates, can support in the design of safe processing and fermentation conditions for plant-based dairy alternatives, which is essential for ensuring safety and stability of this emerging and sustainable food category.

Keywords : spore-formers, plant-based yogurt, Gamma model, meta analysis, *Bacillus cereus*,

## OP02

### Incorporating Biochemical Composition into Predictive Growth Models for Plant-Based Milk products

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Plant-based milks are gaining popularity, yet their microbial safety and spoilage dynamics remain underexplored. Predictive microbiology models typically describe microbial growth as a function of temperature, pH, and water activity, but food composition itself is rarely considered. This study aims to refine predictive models by incorporating the composition of macronutrients as a new factor.

We studied the growth kinetics of *Bacillus licheniformis* in two commercial almond milks (Almond\_H and Almond\_A) and in coconut milk, and compared the results with those obtained from analogous experiments in a standard growth medium (Brain Heart Infusion, BHI). Growth was evaluated across the temperature range 11–55°C, via three independent replicates per condition, using the traditional pour plating. Two types of temperature distributions, equidistant and clustered, were compared for estimating cardinal growth parameters ( $T_{min}$ ,  $T_{opt}$ ,  $T_{max}$ ,  $\mu_{max}$ ). Additionally, via biochemical profiling, we quantified the effect of macronutrient composition (carbohydrates,

proteins, fats) to determine its influence on microbial growth.

Preliminary results indicate that sigmoidal growth curves were obtained under most conditions in both BHI and the two plant-based milks.. However, at higher temperatures ( $\geq 53^\circ\text{C}$ ), significant variability was observed, particularly near the growth/no-growth boundary. Replicated experiments at 55°C showed inconsistent growth, with bacteria either surviving or dying immediately post-inoculation. Such variability is common in food systems and may be due to bacterial metabolites coming into effect at high temperatures. These findings highlight the need for improved modelling approaches that account for biochemical interactions.

This study also contributes to the development of a database on bacterial kinetics in plant-based milk, aligned with fixed ontologies like ComBase. By integrating experimental data with existing literature along with the composition of the milk, predictive models, therefore microbial safety assessments can be improved.

**Keywords :** Predictive microbiology, *Bacillus licheniformis*, plant-based milk, microbial growth modelling, food safety

## OP03

### Modelling microbial inactivation of spoilage microorganisms as a tool to differentiate thermal and non-thermal effects during pulsed electric field processing of plant-based milk alternatives

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**Introduction:** Pulsed electric fields (PEF) is an innovative, electroporation-based technology that could be used to inactivate vegetative microorganisms in dairy alternatives, but its performance is dependent on several process- and equipment- related factors. This study aimed to describe the PEF inactivation kinetics of spoilage microorganisms in oat and almond beverages and present a mathematical approach for differentiating non-thermal from thermal effects.

**Approach:** *Lactiplantibacillus plantarum* (ATCC 8014 and WCFS1) and *Pediococcus pentosaceus*, were used as model organisms for obtaining the inactivation data in a continuous PEF system fitted with either colinear or parallel chamber, applying 10-24 kV/cm electric field strength, 20-130 kJ/L specific energy, and 16-522  $\mu$ s treatment duration. Time-temperature profiles were collected via a combination of fibre optic measurements and simulation based on the Newton's law of temperature. Separately, thermal inactivation experiments were performed in glass capillaries. The temperature profiles were combined with the inactivation kinetic parameters to estimate the F-values and

the antimicrobial effects arising from thermal energy generated during PEF treatment.

**Results:** In several cases, high decontamination levels ( $\geq 5$  log) in the colinear chamber were achieved by, for example, applying 20 kV/cm, 120 kJ/L, 122  $\mu$ s in oat drinks and 24 kV/cm, 130 kJ/L, 119  $\mu$ s in almond drinks. However, elevated final temperatures were also measured at higher energy inputs. Overall, due to the high maximum temperatures in the colinear chamber, non-thermal effects were not differentiated from the thermal effects beyond 80 kJ/L (e.g.,  $F_{68} = 5.99$  s,  $\log N_0/N$  Thermal = 4.15 for *L. plantarum* WCFS1 in almond drinks). In contrast, the parallel design showed lower thermal effects at equivalent energy levels (e.g.,  $F_{68} = 4.02$  s,  $\log N_0/N$  Thermal = 2.79 in almond drinks at 80 kJ/L) and was more effective at inactivating resistant species at lower energy inputs.

**Significance:** The proposed method could enhance the optimization of PEF processing in milk alternatives, where selecting the right equipment and parameters is essential for achieving the necessary treatment levels to ensure microbial stability while preserving product quality.

**Keywords:** Novel food preservation, pulsed electric fields, plant-based beverages, process optimization, spoilage microorganisms

## OP04

### Application of Cold Atmospheric Plasma (CAP) for fish fillets shelf-life extension: moving from laboratory scale to industrial environment

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Cold Atmospheric Plasma (CAP) is an innovative non-thermal disinfection technology, suitable for perishable foods such as fish fillets. A holistic approach was undertaken to explore the application of CAP during storage and distribution of gilthead sea bream (*Sparus aurata*) fillets, both at laboratory and industrial scale, aiming to assess the effectiveness of CAP under various chill chain stages and actual temperature profiles.

Plasma Activated Water (PAW) was produced using a CAP Helium jet (flow rate 0.5 L/min, nozzle–water surface distance 4.3 mm, peak-to-peak voltage 7.2 kV, 100 kHz) (H<sub>2</sub>O<sub>2</sub> and NO<sub>3</sub>– concentrations set to 30 mg/L and 45.1 mg/L, respectively). PAW was dispersed into deionized water and transformed (frozen) into ice (PAI) (H<sub>2</sub>O<sub>2</sub> concentration: 3 mg/L). Two approaches were studied: 1. the immersion of fish fillets in PAW for up to 5 min and 2. the storage of fish fillets in PAI throughout the whole chill chain. For both cases, microbial and quality degradation of fish fillets were evaluated and each approach was optimized for slower microbial growth and minimum quality effect. The results were applied in industrial scale, for fish fillets immersed in

PAW or stored in PAI flakes, following in both cases the typical industrial chill chain. Microbial and physicochemical quality was assessed throughout the whole storage period up to the consumer refrigerators. To ensure consumers safety, PAW safety was investigated through in vivo toxicity experiments using zebrafish embryos.

A PAW containing up to 30 mg/L H<sub>2</sub>O<sub>2</sub> was selected, as this concentration represented the threshold at which no lethality or discernible morphological abnormalities were observed in zebrafish. Indirect applications (PAW/PAI) significantly delayed microbial growth and quality degradation of fillets, extending fillets shelf life by up to 50% compared to untreated samples. Similar results were obtained for the industrial environment as well, i.e., fillets stored in PAI exhibited a shelf-life increase by 1.5 times compared to conventional ones.

The findings indicate that CAP is an effective, applicable and product-friendly technology for maintaining the quality and extending the shelf-life of perishable foods such as fish fillets, even in real production and distribution conditions.

Keywords : cold atmospheric plasma, fish fillets, shelf-life extension, industrial scale, in vivo toxicity experiments

## OP20

### Inhibition of non-proteolytic *Clostridium botulinum* germination in chilled food: model development and application in food safety

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#### Introduction:

Non-proteolytic *Clostridium botulinum* is a significant hazard in refrigerated, processed foods of extended durability (REPFEDs), as it can grow and produce neurotoxins at refrigeration temperatures. Traditional heat treatments (e.g., 90°C for 10 minutes) ensure 6D reduction in spore viability and inhibit spore germination. However, exploring whether milder heat treatments combined with slightly acidic pH and chilled storage may sufficiently prevent spore germination and then guaranteeing food safety is highly relevant in the context of eco-design of new food products. This study presents the results of a multifactorial predictive model for the inhibition of *C. botulinum* spore germination under different heat treatment and refrigerated storage conditions.

#### Materials and Methods:

An exposure assessment model for *C. botulinum* spore was developed, it included heat-treatment, pH, and domestic refrigerator temperature. The spore germination inhibition was derived from the probability of having a recovery before the time of consumption. The data were gathered from literature. The model was built on R software using 2nd order Monte Carlo to integrate separately uncertainty and

variability. The model was applied to heat treatments ranging from 80 to 90°C (at 10 min) and pH levels between 5.4 and 7.0.

#### Results:

Analysis of refrigerator temperature data from 2005 to 2024 revealed that the median consumer temperature was estimated to 6.1°C (with a 75th percentile of the distribution at 7.7°C). The probability of temperatures exceeding 10°C was estimated to 4.5%. For REPFEDs products with a defined shelf-life of 28 days, the storage time was assumed to be relatively short (median of 5 days) but with a probability of consumption occurring after 28 days of 2%. Under these conditions, heat-treatment of 87°C for 10 min combined with a pH 6.6, or, 82°C for 10 min at pH 6.4 enabled to reach at least 3D of spore germination inhibition. This inhibition adds up to the thermal inactivation.

#### Conclusion:

These findings demonstrate the power of the hurdle effect (chilled conditions, acidic pH) when applied to heat-injured bacterial spores. This approach offers a framework for optimizing shelf-life, pH and heat treatments without compromising food safety.

**Keywords :** *Clostridium*, hurdle, germination, lag time, food safety

## OP21

### Accelerated Shelf Life Testing implementation in predicting the stability of High Pressure processed meat products

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Shelf life of meat products is a critical factor in ensuring both consumer safety and product quality. Traditional methods for determining shelf life are labor-intensive and time-consuming, making it challenging for manufacturers to adapt to market demands. Accelerated Shelf Life Testing (ASLT) methodology offers a viable solution by exposing products to controlled elevated conditions, allowing for faster shelf life predictions.

This study aims to establish ASLT as a predictive tool for the shelf life of commercial HPP-treated turkey-based ham and frankfurter-type sausages. The applied ASLT methodology included products' storage under isothermal (4-18°C) and dynamic ( $T_{eff}$ =3.8 and 9.7°C) conditions. The quality parameters assessed comprised of microbiological analysis [i.e., total mesophilic viable counts (TMVC), *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria, and *Enterobacteriaceae* spp.], pH value, colour, water activity, lipid oxidation, texture analysis profile, and sensorial evaluation. Shelf life of meat products was mainly related to their microbiological and sensorial degradation. The growth of TMVC was adequately described through the Baranyi & Roberts model ( $R^2$ =0.97-0.99), while the sensorial degradation followed a zero-order kinetics. Results indicated that sensory evaluation of meat products led to a slight overestimation of their shelf life compared

to the growth of TMVC, and that the rejection criteria should be mainly based on product microbiological quality. The effect of storage temperature on the maximal growth rate of TMVC and the lag phase duration was well described by the Arrhenius equation, which was applied in three different cases, taking into account data based on i) the whole temperature range investigated, ii) three, or iii) two out of four storage temperatures. The obtained results confirmed ASLT as a reliable and efficient tool that replicates spoilage patterns under accelerated conditions, reducing the time and cost of traditional shelf life studies. However, validation is necessary before generalizing this practical protocol, taking also into consideration possible limitations regarding the spoilage mechanisms of each meat product at elevated temperatures.

Conclusively, integrating validated shelf life prediction models into practical applications paves the way for innovation and competitiveness in meat industry, reducing also food waste and meeting consumer demand for minimally processed products of extended durability.

**Keywords:** high pressure processing; accelerated shelf life testing; primary modelling curves; temperature dependence; meat quality.

## OP22

### Multi-agent Quantitative Microbial Risk Assessment for raw milk cheese: A comprehensive modeling approach from farm to consumer

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Ensuring the microbial safety of raw milk cheese requires a robust, quantitative understanding of contamination pathways and risk mitigation strategies. In this study, we develop an original agent-based Quantitative Microbial Risk Assessment (QMRA) model that explicitly represents farms, animals, and cheese batches, and consumers as individual agents, allowing for a realistic representation of microbial transmission and control measures throughout the production chain.

Grounded in extensive knowledge of the French raw milk cheese sector, the model simultaneously addresses three major pathogens of concern: STEC, Salmonella, and *Listeria monocytogenes*.

By integrating stochastic Monte Carlo simulations, the model accounts for variability in microbial shedding at the farm level, contamination dynamics during milk collection

and cheese processing, and consumer exposure scenarios. This multi-agent approach provides a structured framework that enhances the interpretation of risk scenarios and facilitates risk communication, particularly for risk managers seeking to implement effective, evidence-based food safety policies or consumer recommendations.

By explicitly modeling each component of the system, this methodology bridges the gap between theoretical risk assessment and practical decision-making. It offers a flexible and transparent tool for evaluating microbial risks in raw milk cheese and can be extended to other foodborne hazards and production systems.

The presentation will focus on getting to grips with the model, so that users – be they veterinary inspectors, farmer-processors, industrials or consumer representatives – can use it as a food safety management tool.

Keywords : Farm-to-Fork Approach, One Health, Raw milk cheese

## OP23

### sQMRA-R: a flexible and user-friendly tool for Quantitative Microbiological Risk Assessment of foodborne pathogens

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Quantitative microbiological risk assessment (QMRA) is a useful methodology for the quantification of risk and is widely used in food microbiology. Unfortunately performing a comprehensive QMRA study requires a lot of data, and getting a thorough understanding of the steps involved can be time consuming.

Swift QMRA (sQMRA) [1] was developed for the easy execution of quick explorative QMRA calculations at a reduced data need for any pathogen and food product combination. The model follows the flow of foodborne pathogens on products from retail through preparation and heating, and includes a dose-response step for the estimation of infections and cases of illness. This model was expanded upon in a subsequent version by introducing uncertainty estimates and adding growth during storage at home [2].

While this tool is freely available, it requires Microsoft Excel and @RISK, something not everyone has access to and not everyone is accustomed to using.

To improve availability and accessibility of the tool, we developed sQMRA-R, a reimplementing of sQMRA into the R programming language, along with an easy to use app in R-shiny, a front-end application for R implementations.

This R-shiny application is more user friendly and therefore more accessible to people unfamiliar with programming languages like R and @RISK. Despite the tools almost being functionally identical, there are several advantages to using sQMRA-R compared to sQMRA: 1) a table holding the results for all iterations is available to users, allowing them to explore results without being limited to the summaries presented in the tool, 2) sQMRA-R can be run step by step, allowing users to explore different intermediate results, 3) the R implementation is a lot faster than the @RISK version.

The increased user-friendliness of sQMRA-R makes the tool accessible not only to scientists, but also to policy makers and stakeholders, improving the reach of the QMRA field.

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## OP24

### Microbial Risk Assessment of Ready-to-Eat Fresh Produce

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Fresh horticultural produce, particularly minimally processed leafy greens and fruits, is an essential part of a varied and balanced diet, providing vital nutrients for overall health. However, contamination can sometimes occur, posing potential health risks and contributing to foodborne illnesses. Recycling wastewater is crucial for sustainability and can be used for crop irrigation, but it may carry harmful pathogens. This study conducted a Quantitative Microbial Risk Assessment (QMRA) to evaluate and rank microbial hazards associated with consuming ready-to-eat (RTE) fresh produce irrigated with treated wastewater, covering its entire farm-to-fork lifecycle. A QMRA model was developed to assess human exposure to foodborne pathogens through RTE fresh produce consumption, estimating potential annual infection risks based on a four-step risk assessment framework. The model's input parameters included the initial concentration of pathogens in irrigation water, pathogen inactivation through chlorine treatment, cumulative decay, post-harvest chlorine treatment, cold storage at 4°C, and final pathogen concentration on RTE crops. Literature-based dose-response models were

used to calculate daily and annual risk estimates. Findings indicated that for scenarios where chlorine is applied, the overall risk is low, with *Cryptosporidium parvum* posed the greatest annual risk, followed by *Giardia lamblia* and norovirus, and adenovirus. Sensitivity analysis highlighted RTE fresh produce consumption as the most influential factor in the QMRA model, followed by cumulative decay, potential microbial growth on shelves, cold storage effects, and post-harvest chlorine treatment. This ranking prioritizes *Cryptosporidium parvum*, *Giardia lamblia*, norovirus, and adenovirus (in descending order) as pathogens of concern in RTE fresh produce irrigated with treated wastewater. A higher annual risk ( $P_{\text{annual}}$ ) was observed for RTEFP not treated with chlorine at the pre-harvest (irrigation) and post-harvest (packaging) stages, highlighting the importance of chlorine treatment as a measure which aids to reduce microbial loads and reduce the final  $P_{\text{annual}}$  (1 – 5.7 logs). The study highlights the need for risk-benefit in balancing potential chemical (chlorine) human exposure and the benefits of reduced microbial loads on RTEFP.

## OP25

### Developing a user-friendly risk assessment tool to assess the food safety risks of fresh produce production and landscape use

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Fresh leafy greens and other produce are a billion-dollar industry in California and are concentrated in the Salinas and Imperial Valley growing regions. Outbreaks of foodborne bacterial pathogens due to *E. coli* O157:H7 in people have been traced back to produce grown in these regions, which have led to investigations on the origin of the bacterial contamination in the crop fields. Despite much scientific effort, it is difficult to determine the route of *E. coli* O157:H7 contamination in fresh produce.

The objective of this project is to develop a risk assessment model to assess the food safety risk of pathogenic *E. coli* contaminating fresh produce. To achieve this goal, we conducted the project in three phases, where we: 1) conducted a literature review of the evidence regarding risk factors involved in fresh produce contamination, 2) conducted a series of stakeholder meetings, including focus groups and expert opinion, to define the priorities in risk management and parameterization in a risk assessment model, and 3) developed a framework for risk assessment that integrates different sources of

information, such as literature, expert opinion, and publicly available data, in a user-friendly interface to evaluate where and when the risk of a contamination event is increased.

A total number of 18 experts from different sectors were consulted and their input on the different areas of risk management was integrated into the model. Our results highlight the areas of concern and provide a guideline for prioritizing risk mitigation activities in fresh produce contamination. The model framework is fully open source and developed in R with an interactive user interface using R shiny for accessibility. The developed framework allows for integration of new information as it becomes available and updating and adapting the risk assessment model as needed.

The developed tool is currently in beta testing and will be made available to help growers be more proactive in managing risk from foodborne pathogen intrusion into their fields, contributing to an even safer supply of the produce and leafy green products grown in California.

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Keywords : mixed methods, participatory modeling, expert knowledge elicitation, risk mitigation

## OP26

### Quantitative risk assessment of *Bacillus cereus* in roasted chicken combining predictive microbiology and real data from a major Spanish retailer

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*Bacillus cereus* can survive thermal processes in its spore form and produce toxins under favourable conditions. Its presence in ready-to-eat products like roasted chicken may pose a public health risk if post-process storage conditions allow spore germination and growth. This study aimed to assess the survival, growth, and emetic toxin production of *B. cereus* in roasted chicken marketed by a major Spanish retailer, using predictive microbiology models and real-world data on thermal processing and storage conditions at retail, transport and consumer levels.

Historical microbiological monitoring data from roasted chicken samples were used to estimate prevalence and contamination levels. Thermal inactivation was simulated using the Bigelow model with published parameters for vegetative cells and spores. Time-temperature profiles from large and small ovens, as well as storage scenarios up to 36 h (including hot-holding at retail, transport, and domestic refrigeration), were used to simulate real-world handling. Growth and toxin production were estimated using the approach of Ellouze et al. (2020), based on cardinal temperature values and the Baranyi model.

Simulation outputs based on predictive models showed an effective inactivation of *B. cereus* vegetative cells under all cooking conditions, reaching more than 4 log reductions. Complete spore inactivation was achieved only in large ovens, while small ovens achieved only 1 log reduction of spores, indicating insufficient lethality for spore control. Three storage scenarios, combining retail hot-holding and domestic storage up to 36 h, were evaluated. In all cases, predicted *B. cereus* growth remained below 3 log CFU/g by the end of storage, starting from an initial contamination level of 1 CFU/g. The maximum estimated concentration was 2.76 log CFU/g under the most permissive storage scenario. Cereulide production simulations showed that toxin levels remained below the detection threshold (0.2 ng/g), with toxin formation only predicted under abuse conditions not observed in practice ( $\geq 34.9$  °C for  $\geq 11.6$  h). The integration of predictive modelling and risk assessment supports that *B. cereus* poses a very low risk in roasted chicken under current retail and household conditions. Nevertheless, standardized thermal treatments and minimizing storage at suboptimal temperatures remain essential control measures.

**Keywords :** predictive modelling, quantitative risk assessment, ready-to-eat foods, spore-forming bacteria, toxin production, food safety.

OP27

## Optimization of Conventional Hot-Air Drying of Peaches Using Ultrasonic Pretreatment: Modeling with MATLAB

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This study presents an advanced computational framework for optimizing the hot-air drying of peaches, enhanced through ultrasonic pretreatment, with the modeling and simulation fully implemented in MATLAB. The objective was to improve drying efficiency and moisture transport using a mechanistic modeling approach, supported by real-time data fitting, parameter estimation, and predictive simulation, all conducted within MATLAB's environment. Ultrasonic pretreatment was applied to peach cubes at 150 W for 10, 20, and 30 min, prior to convective drying at 50, 60, and 70°C. Experimental drying data, including time-dependent moisture content, was collected and processed. MATLAB was used not only to store and organize the data but also to construct a complete modeling workflow based on diffusion-dominated moisture transport, governed by Fick's second law. A partial differential equation (PDE) model was implemented in MATLAB to simulate internal moisture diffusion. Numerical solutions were obtained using finite difference methods, with spatial and temporal discretization handled through custom code. The model incorporated variable initial conditions and boundary layer resistances to reflect realistic drying conditions. Key parameters, such as effective diffusivity ( $Deff$ ), were estimated dynamically via inverse modeling techniques. An optimization routine was implemented to

minimize the error between simulated and experimental drying curves, ensuring convergence of  $Deff$  estimates under each process condition. Additionally, MATLAB scripts performed temperature-dependent regression to quantify the Arrhenius relationship between  $Deff$  and drying temperature, with activation energy ( $Ea$ ) calculated accordingly. The computational environment enabled extensive sensitivity analysis, where variations in input parameters (pretreatment time, temperature, slice thickness) were simulated to understand their impact on moisture distribution and drying time. Graphical visualization of drying curves, moisture profiles, and parameter influence was integrated into the MATLAB code, providing a comprehensive and interpretable platform for process engineers. This model-driven approach, fully contained in MATLAB, enables predictive simulation of drying performance across a range of operating conditions, without relying on empirical curve-fitting or thin-layer approximations. The results demonstrate that ultrasonic pretreatment substantially enhances drying performance by increasing  $Deff$ , particularly at higher temperatures. The developed MATLAB framework offers a powerful tool for real-time process analysis, scale-up planning, and digital twin applications in the food processing industry.

Keywords : drying, pretreatment, matlab, diffusion equation

## OP36

### Fluorescence microscopy for directly tracking the proliferation of *Escherichia coli* in baby leaves of cultivated and wild lettuce

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Foodborne outbreaks caused by vegetables contamination with human pathogens, like *Escherichia coli*, often involve salads, which pose a higher risk due to raw consumption. Baby leaves, commonly consumed as ready-to-eat salads, may become contaminated during processing. The objectives of this study were: (i) to monitor the proliferation of *Escherichia coli* in baby leaves of romaine lettuce (*Lactuca sativa* L. var *longifolia*; RL) and wild lettuce (*Lactuca serriola* L.; WL) using fluorescence microscopy; (ii) to assess the effect of plant species and incubation time on single-cell outgrowth and (iii) to estimate the variability in colony outgrowth. The leaf epidermis of both species was placed on top of a medium prepared with plant juice extracted from RL or WL leaves, respectively, and solidified with agar. The samples were then inoculated with 10 µL of *E. coli* S-17-1 pH60, exhibiting green fluorescence, and incubated at 37 °C for 24 h. Fluorescence images were acquired after 5, 30, 120, 480 and 1440 minutes of samples' incubation. Proliferation was quantified by measuring the fluorescence area (mm<sup>2</sup>) and intensity (arbitrary units, AU). Image

analysis was performed using the ImageJ software, with five images per species and incubation time. Data and statistical analyses were performed using RStudio. For both RL and WL, no significant differences in the fluorescence area were detected between incubation time 0 and 30 min, while a significant increase was detected as follows: 30 < 120 < 480 < 1440 min. Incubation time accounted for 73.8% of the explained variability, highlighting its predominant role in influencing the area-by-intensity parameter. Species contributed to 23.7% of the explained variability, indicating that there are significant differences between RL and WL in terms of their area-by-intensity values. The results for area and area-by-intensity showed that incubation time is the major driver of variability, with significant increases over time and consistent patterns across species. RL displayed greater growth potential and variability compared to WL, which exhibited a more constrained growth pattern. This study introduces a novel approach for directly detecting and quantifying *E. coli* replication on leaf surfaces.

**Keywords :** *Escherichia coli*; romaine lettuce; wild lettuce; baby leaves; fluorescence microscopy; single-cell outgrowth; colony variability

## OP49

### From raw to ready: Quantitative Microbiological Risk Assessment of spore-formers in plant-based milks and yogurts.

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The increasing demand for plant-based dairy alternatives necessitates the production of safe plant-based products without compromising quality. However, the presence of spore-forming bacteria in plant-based ingredients poses a potential risk. Depending on the processing methods used for different dairy alternatives, heat-resistant spores may survive, germinate, and grow in the product, leading to spoilage and safety concerns.

This study aimed to develop a quantitative microbial spoilage risk assessment (QMRA) model to quantify the risk of two plant-based dairy alternatives, specifically focusing on the processing of ultra-high temperature (UHT) treated plant-based milks and fermented yogurts.

High heat resistant *Bacillus subtilis* and pathogenic *B. cereus* were selected as model organisms for plant-based UHT drinks and yogurts, respectively. The UHT drink model comprises four key steps: (1) initial contamination of raw materials, (2) homogenization, (3) heat inactivation of spores during UHT treatment, and (4) spore germination and outgrowth during distribution and storage. The yogurt-alternative model follows the same first three steps of UHT drink processing, with

slight modifications: in stage (3) there is a heat treatment at lower temperatures, and there is an additional stage (4) fermentation, which involves dynamic pH reduction, followed by (5) storage. An unacceptable level was defined as the final bacterial concentration reaching a maximum threshold of 7 or 5 log<sub>10</sub> CFU/mL at the end of the process for *B. subtilis* or *B. cereus*, respectively. The results highlighted that the presence of high heat resistant spores in ingredients used to produce UHT drinks and yogurts cannot be ignored. They are either able to grow during storage in case of heat treatment survival in UHT products or increase to too high levels in case the acidification in fermentation is not fast enough. Therefore heat treatment intensity in UHT plant-based drinks and the acidification rate during the fermentation of yogurt-alternatives are critical control points for spore inactivation and growth prevention of plant-based dairy alternatives, respectively. Therefore, in some cases, a hurdle approach may be necessary to ensure product safety and quality. Overall, the QMRA models developed in this study can support risk management decisions for plant-based dairy alternatives across different processing scenarios.

Keywords : Risk analysis, process evaluation, *Bacillus* spp., plant-based isolates, food safety

## OP50

### Application of Microbial Modelling in Artisanal Food Production for Listeriosis Risk Prevention

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Dry-cured fermented sausages have been linked to recent listeriosis outbreaks in Spain, influenced by evolving consumer preferences and production methods. Modelling microbial behaviour, under specific manufacturing conditions such as artisanal, is essential for designing effective risk mitigation strategies.

This study aimed to develop a predictive model for *Listeria monocytogenes* (LM) in salchichón, a traditional Spanish dry-cured fermented sausage, to be applied into a Quantitative Microbiological Risk Assessment (QMRA) by integrating molecular and physicochemical data across various production scenarios.

Challenge tests were conducted using a pre-adapted three-strain LM cocktail in lab-scale artisanal salchichón. The impact of different lactic acid bacteria (LAB) strains as starter cultures was evaluated. Kinetic data were obtained and modelled to be incorporated into a QMRA using a Modular Process Risk Model (MPRM), covering ripening to consumption steps. Monte Carlo simulations estimated shelf-life across scenarios varying in time, temperature, and molecular traits such as virulence factors and antibiotic resistance. A sensitivity analysis was carried out to define strategies for risk mitigation.

LM showed maximum growth rates ( $\mu_{max}$ ) during ripening of 0.007–0.015 log CFU/g/h and lag phases ranging from 20.88h–138.96h, while LAB concentrations reached 3.5–8.64 log CFU/g, depending on the starter culture used. At retail, LM initial contamination levels of 1, 2, and 3 log CFU/g led to mean concentrations of 1.79, 3.61, and 5.15 log CFU/g at consumption, respectively. A shelf-life of ~75 days exceeded the regulatory 100 CFU/g threshold when initial contamination at storage was >1.1 log CFU/g at the foreseeable time-temperature conditions. Consideration of virulence and antibiotic resistance of the LM strains significantly influenced risk estimations. Time and temperature were critical factors, with ideal conditions (4–10°C, 95% of the final products are sold before 720h) allowing LM levels of 1.88 log CFU/g after ripening. Biopreservation strategies, including LAB enhancement, were integrated into the exposure assessment as mitigation tools for artisanal products.

This research provides robust methodologies for food operators and risk managers to assess risks and evaluate efficient strategies to mitigate the incidence of listeriosis associated with artisanal salchichón consumption.

## OP53

### Predictive Modeling of Salmonella Enteritidis Behavior in Sunflower Microgreens Cultivation and Storage

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Due to the high nutritional content as well as the health benefits, microgreens have become increasingly popular. However, ideal growing conditions are also favorable for foodborne pathogens. Therefore, evaluating microbial behavior in fresh products allows for a greater understanding of the pathogen's behavior. This study estimated the parameters for Salmonella Enteritidis during sunflower microgreen cultivation and at the storage after harvesting. *S. Enteritidis* was inoculated into the substrate at 3.6 Log CFU/g in a microgreen system cultivation. The substrate was sampled over seven days, every three hours for the first 24 hours, and daily until harvesting. The microbial counts for microgreens started on the fourth day when they began growing. After seven days of cultivation, the microgreens were harvested and stored for 14 days at 5±0,5 °C. The counts were modeled using the Baranyi & Roberts model for the growth and the Log-linear model and log-linear with shoulder and tail for the survival conditions. Conversely, after 48 hours, bacterial

counts in the substrate declined at 0.02 h<sup>-1</sup>. The survival model showed a good fit ( $R^2 = 0.92$ ). The sunflower microgreens began growing on day 4, marking the first detection of *S. Enteritidis* at 5.39 Log CFU/g. The population gradually declined with a survival rate of 0.65 day<sup>-1</sup>, reaching 4.63 Log CFU/g by day 7. The model showed an excellent fit ( $R^2 = 0.96$ ). During storage, the population remained stable at 4.39 log CFU/g for 166.60 h. After this period, the population decreased at a rate of 0.13 h<sup>-1</sup> until 228 h and then stabilized at 2.74 log CFU/g until the end of the storage period at 336h. The survival model for storage showed a good fit ( $R^2 = 0.90$ ). These results highlight the importance of mathematical models in understanding the behavior of *S. Enteritidis* during the cultivation and storage of microgreens. Likewise, the implementation of food safety strategies and the mitigation of microbial risks can be guided through the application of these models.

**Keywords :** Microscale vegetables; Growth Kinetics; Foodborne diseases; Cultivation System.

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## Part B – OMICS and Data Science

### OP54

## Growth of *Listeria monocytogenes* in goat's pasteurised milk cheese during maturation: Validating data from a milk model medium

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Previous research showed that a strain of *Leuconostoc mesenteroides*, isolated from goat's raw milk cheese, was effective in slowing down the growth and reducing the maximum concentration of *Listeria monocytogenes* when evaluated in a milk model (i.e., heat-treated reconstituted milk); and, furthermore, that the extent of inhibition was dependent on the initial milk pH. The objectives of this study were: (1) to determine whether the growth of *L. monocytogenes* in goat's pasteurised milk cheese during maturation could be approximated from growth data obtained in milk model medium, either in monoculture or in coculture with *L. mesenteroides*; and if so; (2) to model a milk-to-cheese conversion factor (Cf) for *L. monocytogenes* growth rate.

Challenge tests were conducted by inoculating *L. monocytogenes* in monoculture and in coculture with *L. mesenteroides* in goat's pasteurised milk adjusted at initial pH levels of 5.5, 6.0 and 6.5. The process of cheesemaking went on and cheeses were ripened at 12 °C during 12 days. Each experimental growth curve was adjusted to

a pH-driven dynamic model where the microbial maximum growth rate is a function of pH.

As observed in the milk model medium, in coculture with *L. mesenteroides*, the optimum growth rate (optGR) of *L. monocytogenes* in maturing cheese was affected by the initial pH of milk: the lowest rate of  $0.863 \pm 0.042 \text{ day}^{-1}$  was obtained at the initial pH 5.5, in comparison to  $1.239 \pm 0.208$  and  $1.038 \pm 0.308 \text{ day}^{-1}$  at pH 6.0 and 6.5, respectively. Regardless of the initial milk pH, *L. mesenteroides* did not reduce the maximum load of *L. monocytogenes* in maturing cheeses, as it did in the milk medium. By contrary, at the initial milk pH of 5.5, 6.0, and 6.5, *L. mesenteroides* was able to decrease, respectively, 2.2-fold, 1.5-fold and 1.9-fold the optGR of *L. monocytogenes* in both milk medium and cheese, without significant differences between the matrices. Following such validation in goat's cheese, the square-root of milk-to-cheese Cf for *L. monocytogenes* was estimated by linear regression as 0.751 (SE=0.0108), and type of culture (monoculture, coculture) was not found to affect Cf ( $p=0.320$ ).

## OP08

### Application of Machine learning with Food Import Risk Explorer Risk (FIRE) model to support risk-informed program design.

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The Canadian Food Inspection Agency (CFIA) develops regulations and delivers inspection and other control measures to prevent and manage food safety risks to Canadians. Risk-informed decision making is at the core of the organization's work. The CFIA is evolving the way risk is managed and embracing technology to provide more efficient and responsive services.

More than half of Canada's food supply is imported, with both the variety and quantity of imported foods on the rise. The CFIA needs to be able to prioritize and monitor high-risk food/hazards regularly to select the most effective control measures based on risk. To inform these decisions, risk assessment models have been developed. The Food Import Risk Explorer model (FIRE) provides a risk framework to compare health risks from different microbial hazards across food commodities. In addition, Machine learning (ML) models are being used for exploratory/predictive trend analyses for high-risk products.

FIRE is a quantitative, comparative risk assessment model using trade volume, consumption information, and hazard characteristics (dose-response, growth/inactivation, health burden) to estimate risk. FIRE

adopts a hybrid modelling approach using both predictive and calibration-based approaches, linking risk per serving estimates to Public Health Agency of Canada estimates of the burden of microbial illness. FIRE estimates total disability-adjusted life years (DALY) at both the population level (which reflects current consumer preferences), and, on a per serving basis (which reflects inherent risk; foods consumed infrequently or in smaller amounts e.g., spices). The use of these risk metrics provide a holistic view, which further informs food safety decisions by risk managers.

FIRE and exploratory/predictive trend analysis models have been developed for high-risk commodities such as fresh fruits & vegetables. We are adding data on more commodities such as dairy and fish & seafood. However, in commodities such as Manufactured foods, surveillance data is limited, therefore, to estimate the risks from these foods, extensions to the FIRE and ML approaches are needed.

In our presentation, we will present findings from FIRE / ML models and discuss our exploration of approaches to address the data paucity for manufactured foods (expanded ML, and Bayesian methods), and express uncertainty.

Keywords : imported Food Risk Model Machine learning

## OP09

### **“Multivariate Food Predictor”: a tool for non-destructive assessment of microbial spoilage of Meat, Ready-To-Eat meat products, and Fresh-cut salads**

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“Multivariate Food Predictor” is a freeware developed in R (available at [https://skandamis.shinyapps.io/Videometer\\_Predictor/](https://skandamis.shinyapps.io/Videometer_Predictor/)). It accepts multispectral or hyperspectral imaging data (MSI: reflectances at different wavelengths, or FTIR absorbances at multiple wavenumbers), as well as other multivariate data related to food properties, e.g. pH, aw, TVBN, TBARS, color, etc. The software performs normalization, mean centering and smoothing of data through various pre-processing algorithms, such as Standard Normal Variate (SNV), auto/range scale, baseline correction and 2nd derivative Savitzky-Golay smoothing. Dependent variables in the uploaded datasets include microbial population levels (Log CFU/g), the lethality value (F-value) of a treatment, or freshness classes, via ordinal variables, such as, fresh, semi-fresh, spoiled, etc.. Dimension reduction may be performed via PCA, in order for the user to understand the contribution of each independent variable to the explanation of the variance of the dependent variable and to select the minimum variables that explain the maximum percentage of the variance

(factor-reduction/feature selection). The tool provides predictions based on the following machine learning algorithms: (i) PLSR, PLSDA, Random Forest Regression, Linear or Radial Supporting Vector Machines (SVM) and Artificial Neural Networks (ANN), with user defined number of trees or neurons of one hidden layer. A training dataset can also be used to provide predictions against an independent (validation) dataset. Once the training data is loaded, a graphical representation of the dependent variable of all data (with relevant labeling) is given, in relation to all independent variables together with a PCA bi-plot. The data then trains the machine learning algorithms. When the (PLSR) algorithm is used, the weighted coefficients and 95% confidence intervals are calculated with bootstrapping for a user-specified number of iterations. Examples of the tool performance are provided for the prediction of total viable counts of fresh beef, cooked meat products, the freshness classes of fresh cut salads and the process lethality (F-values) of tomato-based products.

Keywords : Multivariate analysis, machine learning, principal component analysis, SVM, PLSR, ANN, spectroscopy

## OP10

### Microbial interactions between starter and adjunct cultures shape the metabolic potential and flavour formation of cheese ripening

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Fermentation of cheese is a complex process in which microbial interactions drastically alter the surrounding environment. Previously, we studied the role of microbial interactions in flavor formation during a year-long Cheddar cheese-making process using a commercial starter culture containing *Streptococcus thermophilus* and *Lactococcus* strains [1]. The results highlight the crucial roles of competitive and cooperative microbial interactions in shaping the cheese flavor profile [1].

In this study, we use a multi-omics integrative systems biology approach to investigate how different adjunct ripening cultures interact with a starter culture and influence the metabolome and final flavor perception of four cheeses over a nine-month ripening cycle. A similar starter culture was used to produce cheeses with different microbial compositions. For the reference cheese, only the starter culture was used, whereas the other cheeses were supplemented with varying combinations of strains, including two compositions specifically enriched with highly proteolytic *Lactobacillus helveticus*.

By analyzing metabolic dynamics in relation to microbial dynamics and functional genomics, we

demonstrate that *Lactobacillus helveticus* plays a crucial role in determining the fate of key resources and flavor-related compounds during cheese-making. Specifically, it limits the utilization of galactose, a carbon source produced by *S. thermophilus*. Moreover, the inclusion of supplementary strains in the *L. helveticus* adjunct culture restores galactose utilization. Within these adjuncts, one specific *Lactococcus cremoris* strain appears to be responsible for converting galactose to ethanol, indicating strain-level variation. This, in turn, increases ethyl ester production, which is typically limited by ethanol availability, enhancing the sweet and fruity flavors associated with such compounds.

Furthermore, sensory and metabolomics analyses of the final products indicate that the increase in free amino acid content due to *Lactobacillus helveticus* activity is an even stronger driver of sweetness and fruitiness. Thus, our study highlights how interactions between starter and adjunct cultures shape the cheese environment, further influencing the community's metabolic potential and the sensory perception of the cheese.

[1] Melkonian, C., Zorrilla, F., Kjærboelling, I. et al. Microbial interactions shape cheese flavour formation. *Nat Commun* 14, 8348 (2023). <https://doi.org/10.1038/s41467-023-41059-2>

## OP11

### Multi-target prediction with deep neural networks in foodomics: a case study on *Brochothrix thermosphacta* to predict volatile organic compounds linked to fresh meat spoilage

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The microbial spoilage of meat is generally associated with the generation of volatile organic compounds (VOCs) and off-odors. While predictive modeling benefits shelf-life determination, predicting microbial metabolism is very complex. This is because many real-world problems are characterized by multiple related variables (e.g. multiple VOCs linked to meat quality) but traditional supervised learning mostly focuses on predicting one single target. Therefore, this study introduces the concept of multi-target prediction (MTP) for this type of tasks. Particularly, this method is demonstrated by a case study about predicting VOCs produced by *Brochothrix thermosphacta* on pork simulation media under various packaging atmospheres.

The used dataset comprises the total plate counts (TPC), O<sub>2</sub>/CO<sub>2</sub> ratios, and real-time VOC data (i.e. concentrations of 37 VOCs) of 840 individual media samples inoculated with *B. thermosphacta* and stored under 20 atmospheres (O<sub>2</sub>: 0%-70%, CO<sub>2</sub>: 0%-60%, N<sub>2</sub>: rest) for different times. The feasibility of MTP is tested by predicting several microbial VOCs with both regression and classification models. Since some of the compounds can be linked to each other via pathways, a two-branch neural network

(2BNN) inputting different types of data (TPC, %O<sub>2</sub>/CO<sub>2</sub>, metabolism information for each VOC) is proposed as the first attempt in the food domain. A new Python package called DeepMTP is used. The models are trained and validated on data from all possible selections of 19 atmospheres and finally tested on the remaining atmosphere.

MTP successfully works in this case study because of the close relationship between microbial quality and VOC production. Based on TPC and other additional inputs, the 2BNN models can deliver accurate classification for different VOC production levels. In contrast to a conventional MTP method (without the input of metabolism information), the RMSE values decrease by more than 50% when using 2BNN to predict VOC concentrations.

The present research explores the possibilities of MTP in predicting meat volatolome and thus enriches the content of predictive modeling in food science. To implement MTP in different omics studies, it would be important to first define a suitable research question and then determine appropriate model settings for achieving meaningful predictions.

## OP12

### ResPathExplorer: A Python-Based Library for Pathway Analysis and Resistance Gene Mapping through KEGG and CARD Integration

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Python libraries are collections of modules and packages that combine pre-developed functions for specific tasks, promoting code reuse, speeding up development, and eliminating the need to rewrite analysis pipelines from scratch. The Kyoto Encyclopedia of Genes and Genomes (KEGG) integrates genomic, chemical, and systemic data and offers various bioinformatics tools for the integrated analysis of metabolic pathways, genetic regulation, and molecular interactions, facilitating the understanding of complex biological processes. The Comprehensive Antibiotic Resistance Database (CARD) summarizes data on genes, proteins, and phenotypes related to bacterial resistance, making it a remarkable source for analyzing antibiotic resistance. In this work, we developed

ResPathExplorer, a python-based library designed to interpret transcriptomic data, identify metabolic pathways enriched in a set of genes, and map the prevalence of antibiotic-resistant genes. Integrated with KEGG and CARD, this library transforms a simple list of genes into graphical representations of enriched pathways, mapping genes in metabolic processes and their correspondence in the CARD database. ResPathExplorer is a valuable tool for analyzing biological and chemical risks in various research areas. In the alimentary sector, its application can contribute to the characterization of pathogenic bacteria in food, helping to monitor antimicrobial resistance and build safer, more resilient, and sustainable food systems.

Keywords : bioinformatics, python, gene expression, metabolic pathways, resistance genes, enrichment

## OP25

### Leveraging AI for Advanced Querying and Visualization of Microbiological Data: The New Pathogens-in-Foods Database

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The growing volume of incidence data on biological hazards in foods necessitates advanced tools to extract actionable insights and support decision-making in food safety. The Pathogens-in-Foods (PIF) database, a platform dedicated to cataloguing the occurrence of pathogenic bacteria, viruses, protozoans and nematodes in foodstuffs, contains so far a total of 13100 data entries, and would certainly benefit of an intelligent system for data exploration and analysis. The present work focuses on developing an intelligent agent for PIF, designed as a chatbot-based Visualization-oriented Natural Language Interface (V-NLI), which enables users to query the database, perform meta-analyses, generate dynamic reports, and

visualize compiled data. Leveraging a small language model (e.g., Mistral or Llama 3.1) combined with Retrieval-Augmented Generation (RAG), the system has been fine-tuned to provide accurate, context-aware responses. This innovation aims to improve response times for database queries, and offer customized analyses tailored to the needs of researchers, academia, regulators, and food safety agencies. By automating complex data extraction and visualization tasks, the intelligent agent aims to enhance the usability and adoptability of the PIF database as a web resource that contributes to supporting both food microbiological research and more informed decision-making in food safety management.

**Keywords :** foodborne pathogens, intelligent agent, natural language interface, data visualization, meta-analysis, microbiological safety

## OP33

### Predictive Modeling of Curcuminoid Bioaccessibility in Complex Food Matrices via Machine Learning

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**Background:** Understanding how effectively bioactive compounds are absorbed is essential for evaluating the nutritional value of various food products. Despite its importance, the influence of food matrix composition on this absorption process remains underexplored due to the complexity of the task. To investigate this further, this study focuses on curcuminoids—bioactive substances derived from *Curcuma longa*—which are well-known for their potential health benefits but also for their limited bioavailability.

**Project Objective:** The primary aim of this research is to examine how different food matrix compositions affect the bioaccessibility of curcuminoids, a critical determinant of their absorption. A secondary goal is to develop predictive models that can guide the design of functional foods and help consumers better understand the impact of food composition on nutrient uptake.

**Methods:** Static in vitro digestion procedures were carried out based on the standardized INFOGEST protocol to simulate gastrointestinal conditions for curcuminoid-enriched food products. Bioaccessibility was quantified using High-Performance Liquid Chromatography (HPLC) and UV-Vis spectroscopy. Various food formulations—mainly custards and biscuits—were created using dietary fiber supplements

(including both soluble and insoluble types) along with other nutrients. These formulations provided a dataset for developing predictive models that link bioaccessibility to the physicochemical properties of the food matrices, such as nutrient composition, texture, ingredient density, and moisture retention.

**Results:** Macronutrient composition (i.e., combined protein, fat, and carbohydrate content) was identified as a key predictor of curcuminoid bioaccessibility. This variable was incorporated into a Bayesian hierarchical model, which demonstrated strong predictive accuracy ( $R^2 = 0.97$  for optimized performance and  $R^2 = 0.93$  under leave-one-out cross-validation). These promising findings suggest that stochastic modeling could be a valuable approach for analyzing how various food matrix characteristics influence the absorption of bioactive compounds.

**Conclusions:** This study provides a conceptual framework showing that the complex relationship between food matrix characteristics and nutrient bioaccessibility can be effectively modeled. The results set the stage for more advanced research that could inform both food formulation strategies and consumer guidance, potentially leading to enhanced dietary health outcomes.

**Keywords :** food matrix, bioaccessibility, systematic screening, probabilistic modelling, Machine Learning

## OP34

### An In-Silico Prediction Pipeline for Data Mining of Antifungal Peptides for Potential Applications as Food Preservatives

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Globally consumed bread deteriorates fast mostly because of mold contamination. This not only impacts consumers but also leads to significant economic losses in the baking industry. Fungal development on bread also poses health risks due to potential mycotoxin production. Traditionally, chemical preservatives such as calcium propionate have been used to counteract this issue. However, concerns regarding their safety and potential for microbial resistance have spurred research into natural alternatives, also due to general sensory dissatisfaction and low awareness in the food market. This project aims to develop novel solutions for bread bio preservation based on the exploitation of bioactive peptides and plant antimicrobial compounds.

Multiple databases are available where bioactive peptides from different sources are classified based on antifungal, antimicrobial, antioxidant, antihypertensive, etc. A selection pipeline was made for the peptides on the following parameters – sequence length (cutoff at 5-30 amino acids), net charge, hydrophobicity (using

PTPAMP), amphipathicity (using antip), haemolytic activity (using tools like happen and hemopred), Gibbs free energy (using  $\Delta G$  predictor), and MIC (using antifungipept). The dataset collected using the above parameters were visualized using heatmap and k means clustering to choose the potential antifungal peptides. The peptides were sequenced aligned using Multalin tool for identification of similar amino acid residues and subjected to key motifs discovery using MEMESUITE to highlight specific characteristics.

This pipeline was also used for selection of plant matrices to extract bioactive peptides and other antimicrobial compounds using enzymatic treatment. In vitro antifungal assays such as hyphal radial growth test, spore germination count, growth kinetics were set up to screen for activity against indicator fungus *Penicillium roqueforti* and other molds frequently isolated in bakery environments. After screening, the selected compounds would be applied in bread recipe for long term shelf-life test as novel preservatives.

Keywords: antifungal, peptides, antimicrobial compounds, plant, bread.

## OP37

### Structural Modeling of Antimicrobial Peptides from Lactic Acid Bacteria: Insights into Conserved Motifs and Functional Diversity

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Among the food-grade antimicrobial peptides (AMPs) used in the European Union, nisin (E234) stands out for its broad-spectrum activity. Nisin-like AMPs are typically sought from natural food products, especially fermented foods, by screening the genome of indigenous microorganisms, primarily by computational tools that predict their sequence composition and model their structural conformations.

This study compared the three-dimensional structure models of six AMPs (Nisin A, Nisin Z, Lactocin S, Pediocin PA-1, Divergicin 750, and Carnobacteriocin A) from the LABiocin (<https://labiocin.univlille.fr/>) database, which were reported in lactic acid bacteria (LAB) isolated from meat or meat products. The sequences of selected AMPs were obtained in .fasta format from the NCBI database. Sequences were subjected to multiple alignments with the CLUSTAL W algorithm and visualized using the Jalview software, where the leader peptide and the mature peptide sequence were obtained. Three-dimensional models of selected AMPs were retrieved from the AlphaFold Protein Structure Database in .pdb format, and structures were visualized using the Chimera X software.

Results showed a conserved region between Nisin A, Nisin Z, and Lactocin S, composed of the amino acid sequence FNDLV, which, after visual analysis, was found to be located in distinct positions within the three-dimensional space of the peptides. Class II conserved amino acids, also known as pediocin-box (YGNGV), also exhibited distinct spatial arrangements within the three-dimensional structures of the different class II AMPs: Pediocin PA-1, Divergicin 750 and Carnobacteriocin A. This variation suggests that although the sequence is conserved, the region's spatial positioning and orientation can differ from one peptide to another.

Such differences in the three-dimensional conformation could influence how AMPs interact with their target receptors, potentially affecting their antimicrobial activity and specificity. Given the varied nature of the predicted structures of the AMPs analyzed, strategies to enhance and potentiate their antimicrobial activity can be further developed. These findings underscore the importance of structural modeling in predicting AMP efficacy and suggest new avenues for the rational design of peptide-based food preservatives.

**Keywords :** 3D modelling; Biopreservation; Bioactive peptides; Predictive modeling; Food safety

## OP38

### Preparing for the next generation QMRA

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In this era of explosive technological innovation, it is imperative to integrate omics information and artificial intelligence into Quantitative Microbiological Risk Assessment (QMRA) to enhance its accuracy and timeliness, thereby reducing uncertainty. This presentation will showcase our work on the next-generation QMRA. We will first introduce a case study on the rapid detection of different serotypes of *Listeria monocytogenes*, demonstrating the potential of digital PCR methods to improve hazard identification and exposure assessment. Next,

we will explore the application of Genome-Wide Association Studies (GWAS) in *L. monocytogenes*, highlighting its pivotal role in integrating omics information into QMRA by bridging genomic and phenotypic data. Additionally, we will delve into the use of machine learning techniques in modeling the growth of *L. monocytogenes*, with a particular focus on addressing challenges posed by small or imbalanced datasets.

## OP42

### AI-Driven System for Microbiological Alerting and Pattern Detection in the Pathogens-in-Foods Database

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The growing scale and complexity of microbiological food surveillance data demand intelligent systems for efficient data exploration and pattern identification. This work introduces an AI-based alerting and analysis system built for the Pathogens-in-Foods (PIF) database - a harmonized collection of microbial prevalence data in food products across Europe. The system is designed to help researchers detect unusual patterns, uncover hidden associations, and surface early alerts on biological hazards in food. We approach the challenge as a pattern detection task grounded on microbial prevalence profiles and similarities across food-pathogen combinations. The system leverages collaborative filtering algorithms such as K-Nearest Neighbors (KNN), along with matrix factorization methods including Singular Value Decomposition (SVD) and Non-negative Matrix Factorization (NMF), to identify structural

patterns in the data. Cosine similarity was used to create food and pathogen similarity matrices, enabling the system to detect links between foods with similar microbial profiles, and a rank-based alert model using weighted prevalence was implemented to support identification of high-priority signals. The system effectively highlights atypical patterns, clusters of similar foods, and high-prevalence microbes, supporting exploratory workflows and early warning use cases. This AI-enhanced alerting tool demonstrates how pattern detection and data mining techniques can unlock new insights from structured surveillance datasets. By surfacing significant trends in the PIF database, the system offers a practical resource for researchers and food safety stakeholders aiming to improve microbial monitoring and data-informed decision-making in Europe.

**Keywords:** microbial prevalence, data mining, food safety, PIF, pattern detection, matrix factorization

## OP43

### How to create healthy aquatic food systems and safe seafood in the context of increasing global temperatures and extreme weather phenomena

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With increasing global temperatures and extreme weather events such as hurricanes and floods, the growth and spread of seafood spoilers and foodborne pathogens, including antimicrobial resistant bacteria (ARB), become more likely to occur. The development of intelligent approaches to predict the increase of such bacteria in aquatic environments (particularly aquaculture and shellfish farms facilities) and subsequently the increased prevalence in seafood to tackle the consequences to the public health is required.

Our aim was (i) to identify spoilers and pathogens including ARB using omics (i.e. metagenomics and metabolomics) in farms facilities and seafood (pre- and post-harvest of farmed fish and shellfish) and (ii) to study the growth of isolated bacteria in model seawater systems and model seafood under various temperatures and antibiotic pressure, in order to collect data for determining (through microbiological and analytical approaches) and predicting spoilers and pathogens presence and behavior, including ARB in the seafood value chain. The collected data of the most important pathogens, spoilers and ARB were fitted to the primary model of Baranyi and Roberts (1994). Secondary models

will be further used to assess the effect of various temperatures on the kinetic parameters of such bacteria in both aquatic environment and seafood.

Potential pathogens e.g. *Aeromonas eucrenophila*, *Enterococcus faecium*, *Morganella morganii*, *Providencia rettgeri*, *Stenotrophomonas maltophilia*, and *Serratia marcescens* were isolated from aquaculture facilities and fish, while others e.g. *Escherichia coli*, *Enterococcus* spp. and *Shigella flexneri* from shellfish farms. Seafood spoilers such as *Pseudomonas* and *Shewanella* were also found in handling and processing facilities. In most samples from both aquatic farm systems, a significant number of ARB was detected, including strains with multiple antibiotic resistance (e.g. Streptomycin and Penicillin G). Of them, ARB and non-ARB presented different kinetic parameters in some cases. The findings will be used to develop quantitative microbial risk assessment (QMRA) models and in a couple of intelligent methods using artificial intelligence technology, the rapid assessment of safety and quality of foods will be feasible in the context of the great climate challenges of the 21st century.

key words: aquatic food systems, seafood, Antibiotic-Resistance Risk Assessment

## OP44

### Estimation of the Size of Foodborne Outbreaks Based on Human Genomic Surveillance Data

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**Introduction :** Whole genome sequencing (WGS) offers a useful tool to identify genetic bacterial clusters. Due to the widespread use of WGS for surveillance of foodborne infections in humans, increasing numbers of these clusters are found, and as a consequence more potential outbreaks are recognized than before. As resources are limited, it is not possible to follow up on all these potential outbreaks, and the most relevant ones have to be selected in a reliable and efficient way. Therefore, we need a method that informs on the estimated real outbreak size, based on the size of the identified genetic clusters among the sampled isolates.

**Methods :** We present a method based on probability theory that provides operationally relevant statistics about the size of a genetic cluster, including an estimate of the real cluster size in the population, the uncertainty of the estimate and the probability that the real size exceeds a predefined critical threshold. We explore how these statistics change with the number of sequenced isolates out of the total number of reported infections.

**Results :** We show how our method and findings can be used on sequenced isolate data obtained from human surveillance i) to quantify the real size of a foodborne outbreak with a predefined certainty; ii) to follow the evolution of the outbreak; iii) to determine the number of isolates required to detect outbreaks of a predefined critical size with desired certainty. In simulated scenarios and real-life examples of previously identified genetic clusters of human campylobacteriosis in Denmark, we illustrate how operational questions of risk managers can be answered.

**Discussion :** The developed method can be used to support decision making on the identification of the foodborne outbreaks that require an intervention, and to back up communication to authorities, the industry and the public. Additionally, it can be applied to set up an efficient WGS-based surveillance system of foodborne infections by assisting risk managers in making a balanced choice between the probability of (not) identifying a relevant outbreak and the resources invested in sequencing isolates. Future research should indicate how data from sequenced food isolates can be integrated in this approach.

**Keywords :** WGS; foodborne outbreaks; risk management; human surveillance data; uncertainty

## OP45

### Machine Learning-Based Analysis of Climate Trends and Foodborne Illness Risks in Europe

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Climate change has emerged as a significant driver of public health risks, particularly in the context of foodborne illnesses. This study explores the temporal relationships between climate factors, such as temperature, precipitation, and extreme weather events, and the incidence of foodborne illnesses across the European Union from 2014 to 2024. Climate datasets from the Copernicus Climate Change Services were collected alongside illness incidence reports from the European Centre for Disease Prevention and Control (ECDC) to identify temporal trends, patterns and correlations. Monthly mean and maximum temperatures, precipitation levels, and extreme weather indices were extracted and correlated with the incidence rates of key foodborne illnesses from pathogens, such as *Salmonella* spp., *Campylobacter* spp., and *Escherichia coli*. Advanced machine learning algorithms, like recurrent Neural Networks, were then trained according to these data, and further used to make predictions using future climate projections.

Preliminary results demonstrate that rising temperatures and increased precipitation significantly elevate the risk of pathogen

proliferation in food production and supply chains. More specifically, the data indicate an obvious yearly periodicity where both temperature and foodborne illnesses reach a maximum during the summer months, with the peak of foodborne illnesses predictably following 1-60 days after the peak of temperature, depending on the pathogen. *Campylobacter* had the shortest temporal difference (less than 30 days between the highest temperature and the most reports of cases), while *Salmonella* had the longest (30-60 days).

The study highlights the importance of temporal resolution in understanding these dynamics. Recurrent Neural Networks were able to capture long-term trends in the temporal patterns of climate-related factors. To achieve this, cross-disciplinary approaches in predictive microbiology are necessary to address the challenges posed by climate change. By combining environmental and epidemiological insights, actionable knowledge can be provided to support policymakers, food safety authorities, and public health agencies in mitigating the impact of climate change on foodborne illness risk.

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Keywords : risk assessment, climate change, machine learning, *Escherichia coli*, *Salmonella*, *Campylobacter*

## OP48

### Pilot study to predict the occurrence of foodborne pathogens in milk microbiome testing the animal sewage microbiome in a dairy cattle farm

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In 2022, the number of foodborne outbreaks in Europe increased by 43.9%, highlighting the urgent need to enhance surveillance systems and develop predictive tools for outbreak prevention. Monitoring foodborne pathogens in wastewater has emerged as promising and cost-effective approach to improve foodborne outbreak surveillance. Shotgun metagenomic analysis of human sewage has already proven successful in mapping the circulation of biological hazards and antimicrobial resistance genes within specific regions.

In this pilot study, we explored the feasibility of analyzing animal sewage from a dairy cattle farm to predict the milk microbiome and potentially prevent the entry of foodborne pathogens into the milk and cheese production chains.

A total of four sewage samples and four milk samples, of 50 ml each, were collected from the same farm during four longitudinal samplings conducted monthly between October 2023 and January 2024. Total DNA was extracted from all samples using a mechanical cell disruption followed by the PowerFood® Microbial DNA Isolation Kit. The extracted DNA was quantified

using a BioSpectrometer® and subsequently fragmented and tagged with sequencing indexes and adapters using the Nextera XT DNA Library Preparation Kit. Shotgun metagenomic sequencing was then performed using the NextSeq 500 in paired-end mode (2x150 bp). The sequencing output ranged between 2.75 to 3.80 Gbp per samples. The alpha diversity index, showing the microbiome richness, varied across sewage samples, while it was homogenous for bulk milk samples. Beta diversity analysis clustered all the milk samples together while clustering of the sewage samples was in the same area of the PCoA plot. The predominant genera in sewage included Bacteroides, Prevotella, Alistipes and Pseudomonas. The prevalent genera prevalent in milk included Staphylococcus, Streptococcus Anaplasma and Enterococcus. Correlation analysis revealed a strong association ( $R > 0.88$ ) between specific bacteria species present in both sewage and milk samples, including Staphylococcus aureus which is a relevant foodborne pathogen in the dairy food system.

#### Acknowledgment

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Keywords : microbiome, dairy cattle farm, sewage, foodborn pathogens, shotgun metagenomics

## OP51

### Machine learning-powered uropathogenic *Escherichia coli* (UPEC) growth model and microbial exposure assessment for evaluating the consumer risk of UPEC from ready-to-eat (RTE) pork in Taiwan

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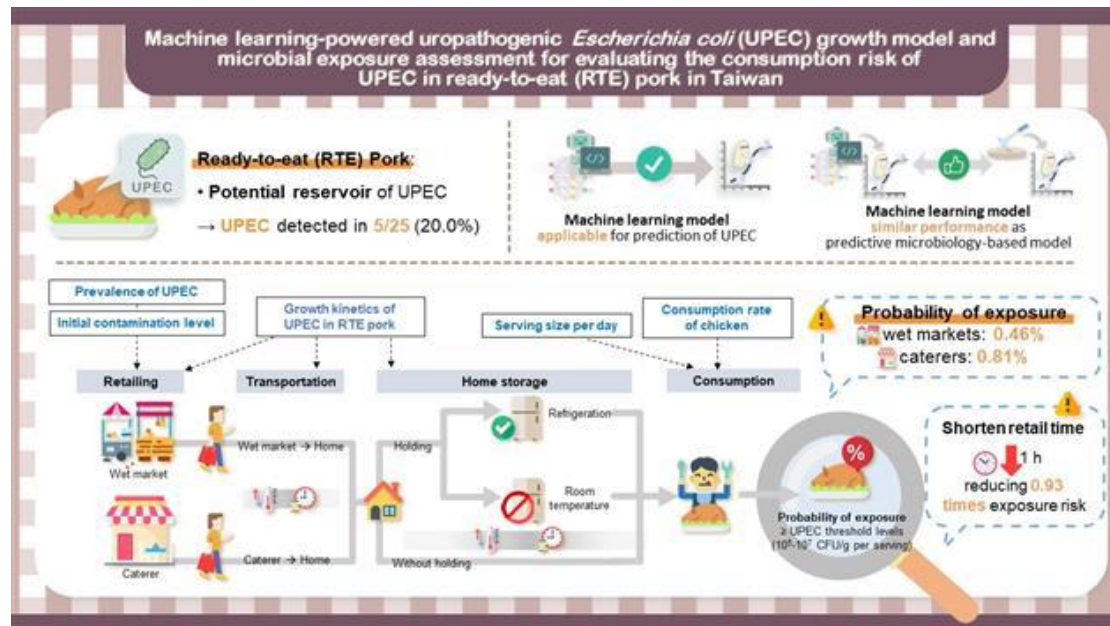
Uropathogenic *Escherichia coli* (UPEC) is linked to foodborne UTIs, with ready-to-eat (RTE) pork as a potential reservoir. RTE pork, often consumed without reheating, is susceptible to contamination during retailing. Machine learning (ML) algorithms can identify microbial growth patterns without predefined relationships. This study aimed to (i) model the growth kinetics of UPEC in stewed pork under varying storage temperatures using a predictive microbiology (PM) and ML-based model and (2) establish a retail-to-fork microbial exposure assessment to evaluate UPEC risk from RTE pork and propose evidence-based interventions.

Twenty-five RTE pork samples from Taiwan's wet markets and caterers were analyzed for *E. coli* using plate count methods, with UPEC identified via PCR (C3509, chuA, C3686 genes). Growth modeling involved inoculating stewed pork with UPEC cocktails (3–4 log CFU/g) at 4–40°C. UPEC population changes were analyzed using IPMP2013 to estimate growth parameters, while observational data were used to train multiple ML models—artificial neural networks, random forests, and support vector machines. Model performance in predicting bacterial counts was assessed using root mean square error (RMSE) and proportion of prediction error (pPE). Exposure probabilities exceeding threshold ( $10^5$ – $10^7$  CFU/g per serving) were estimated by Monte

Carlo simulation (10,000 iterations) using R ('mc2d' package). Input parameters included UPEC prevalence, concentration, growth during retail-to-consumption, time-temperature conditions, and serving sizes. Critical control points were identified through sensitivity analysis.

Results showed that *E. coli* was detected in 9/25 (36.0%) samples, with UPEC in 5/25 (20.0%). Growth models were developed and validated under fluctuating temperatures based on the PM model and ML model. ML model illustrated goodness-of-fit, with RMSE and pPE values indicating reliable predictive accuracy, similar to the PM-based model. The estimated mean probability of high UPEC exposure is 0.46% for wet markets and 0.81% for caterers, with retail-time UPEC concentration being the most significant risk factor. Reducing retail time by one hour lowered exposure risk by 0.93 times, emphasizing the importance of targeted interventions.

Our findings illustrated that ML algorithms serve as a feasible alternative for predicting UPEC growth, providing a framework to mitigate UPEC risks in RTE pork in Taiwan and enhance consumer safety through improved retail practices.



## OP57

### Addressing metagenomic data compositionality and confounding factors in clinical studies for the safety assessment of human microbiome perturbations

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As there are no clearly defined endpoints for healthy human microbiomes, safety assessments of new technologies designed to perturb microbiomes, such as prebiotics, probiotics, or postbiotics, often rely on assessing the changes induced in longitudinal clinical studies [Metris et al., 2024]. Comparing microbiomes measured by sequencing data, such as 16S rRNA, remains challenging because sequencing data are compositional [Gloor et al., 2017], and microbiomes are affected by many confounding factors, such as individual host variability. Many statistical parameters have been proposed to characterise microbiome composition. However, these are based on epidemiological notions, and it is not clear which ones should be considered for risk assessments. We illustrate in some examples, including the gut microbiome, which statistical parameters may be useful for risk assessments and highlight

potential pitfalls. For instance, beta-diversity is useful for understanding confounding factors, while alpha-diversity helps with sample quality control and potentially highlights changes in richness or evenness, which, in some cases, have been linked to unhealthy states. Differential abundance analyses could help identify decreases in commensals or increases in taxa associated with adverse host health conditions, but methods minimising false positives need to be sought and do not completely replace more quantitative measurements.

We discuss the requirement for quantitative measurements alongside sequencing data and host metadata to assess associations of microbiome changes with host response. Additionally, we explore the potential of AI approaches for dealing with confounding factors [Metris et al., submitted].

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## Part C – Modelling Food Microbiome

### OP13

#### Predictive Modelling of *Escherichia coli* and Lactic Acid Bacteria Growth in Fresh sheep Cheese

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The traditional Maltese cheese Ġbejna is characterized by its soft to semi-soft texture and is typically produced from raw sheep's milk without starter cultures. However, the risk of contamination by foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli* during processing and post-processing presents a major public health concern. This study aims to develop predictive models describing the growth and survival dynamics of *E. coli* and LAB in sheep cheese (Ġbejna) under different time-temperature conditions.

LAB isolates exhibiting in vitro anti-*E. coli* activity were selected for assessment in freshly prepared Ġbejna cheese. Three cheese groups were prepared: (1) control cheeses containing LAB (*L. plantarum* A9, *L. plantarum* P8, *L. pentosus* YW11, *L. paracasei* MG5189, *L. fermentum* DM075, *L. brevis* NWAUFU1525, *L. paracasei* OR22, and *C. halodurans* TWMW1.1920), (2) control cheeses inoculated with *E. coli*, and (3) co-inoculated cheeses with both LAB and *E. coli*. Fresh cheese (moisture:  $73.30 \pm 1.47\%$ ; pH:  $6.56 \pm 0.12$ ) was stored at temperatures (4°C, 13°C, 20°C, 25°C, 30°C, 37°C and 42°C). The generalised Lotka-Volterra model incorporating logistic growth was used to characterise

bacterial growth in single and mixed cultures. The dependency of the intrinsic growth rates and lag phases on temperatures is described with a modified Ratkowsky model and calibrated through a multi-experiment parameter estimation approach. After successfully recovering the microbial culture dynamics, model growth kinetics of *E. coli* and all the studied LAB strains were assessed independently and in co-cultures. For example, the growth parameter estimates of *L. plantarum* A9 resulted in a growth rate of  $0.570 \pm 0.001/\text{h}$  with  $T_{\text{opt}}=34^\circ\text{C}$ ,  $T_{\text{min}}=0.77^\circ\text{C}$  and  $T_{\text{max}}=48.12^\circ\text{C}$ . The maximum growth rate of *E. coli* was determined at  $0.779 \pm 0.001/\text{h}$  while temperature parameters were found to be  $T_{\text{opt}}=35^\circ\text{C}$ ,  $T_{\text{min}}=0.74^\circ\text{C}$  and  $T_{\text{max}}=67.7^\circ\text{C}$ . The inhibitory capacity of the LAB strains was also validated by the co-culture modelling approaches. Characterising the specific growth parameters using single and mixed culture data ensures a more accurate estimation of the interspecific interactions occurring in mixed cultures in actual food products. The presented findings provide insightful information on modelling microbial dynamics while performing challenge tests in the actual food products.

Keywords : Predictive modelling; Lactic acid bacteria; Antibacterial; Cheese

## OP14

### Natural Antimicrobial Strategies for Cultivated Meat: Predicting Salmonella Inactivation Through Physiochemical and Formulation Parameters

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Microbial safety is a critical consideration in cultivated meat production, where sterile environments must be maintained to prevent contamination from foodborne pathogens such as *Salmonella* spp. Unlike conventional meat, cultivated meat is grown in bioreactors under controlled conditions, reducing external contamination risks. However, microbial hazards can still arise from raw materials, bioprocessing conditions, and post-harvest handling. This study develops a predictive model for *Salmonella* inactivation in a simulated cultivated meat medium, assessing the effects of oregano essential oil (OEO), pH, temperature, and water activity (*a<sub>w</sub>*).

A Doehlert matrix design was employed to optimize the experimental parameters, with OEO concentrations ranging from 0 to 0.3% (v/w), pH levels between 4.7 and 6.8, *a<sub>w</sub>* values from 0.88 to 0.93, and storage temperatures spanning 8 to 20°C. A *Salmonella* spp. cocktail was inoculated

into the medium, and survival curves were analyzed using Weibull models. A response surface (RS) model, based on a quadratic polynomial equation, was applied to predict inactivation trends under the combined effects of these variables. The RS model demonstrated high predictive accuracy, with RMSE values of 0.14 for  $\delta$  and 0.09 for *p*, and %SEP values of 13.32% and 8.60%, respectively.

These findings highlight the significance of formulation parameters in microbial control strategies for cultivated meat. The study provides a framework for developing scalable, nature-derived antimicrobial interventions, ensuring the microbiological safety of cultivated meat while reducing reliance on synthetic preservatives. Further research should explore synergistic approaches combining bioactive compounds, process engineering, and real-time microbial monitoring to advance food safety in the emerging cultivated meat industry.

**Keywords:** Cultivated meat, microbial safety, *Salmonella* inactivation, predictive modeling, essential oils, food safety

## OP29

### Thermal Resistance of *Geobacillus* spp. in Oat-Based Beverages: Predictive Modeling for Food Safety

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Thermal inactivation kinetics of *Geobacillus* spp. in oat-based beverages were evaluated to establish predictive models used for thermal processes determinations. The study focused on three products: red fruit oat beverage (pH 4.65 - acid food), cocoa and cappuccino oat beverage (pH 6.48 and 5.55, respectively - low acid food), each containing distinct spore-forming bacteria, isolated from formulated beverages (before heat treatment) being the most heat resistant in each product. Heat resistance parameters (D and z values) were determined at temperatures ranging from 100°C to 130°C using the capillary tube method, in an oil temperature-controlled bath ( $\pm 0.1^\circ\text{C}$ ).

For *G.stearothermophilus* spores (DA) in red fruit oat beverage (pH 4.65), D linear values ( $R^2 > 0.96$ ) at 100, 110, and 120°C were 1.04 min, 0.34 min, and 0.12 min, respectively, with a calculated z-value of  $21.52^\circ\text{C}$  ( $R^2 = 0.99$ ), indicating higher resistance even in acidic conditions. In cocoa oat beverage (pH 6.48), *G.thermoglucosidasius* spores (I1B) exhibited non-linear inactivation (Geeraerd with shoulder model) with time to the first log reduction = 3.25 min at 110°C ( $R^2 = 0.99$ ) and 0.98 min at 115°C ( $R^2 = 0.99$ ). For 121°C, linear model was obtained and D-value = 0.3 min

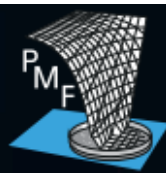
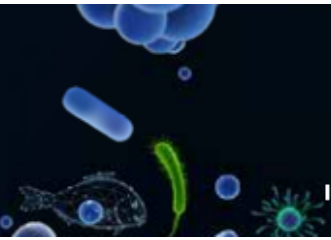
( $R^2 = 0.99$ ), with a z-value of  $10.66^\circ\text{C}$ ; demonstrating enhanced heat resistance compared to red fruit oat beverage. For cappuccino oat beverage (pH 5.55) contained *G.stearothermophilus* spores (I2A), was obtained D-value = 1.33 min at 105°C ( $R^2 = 0.99$ ). At 110°C and 120°C, data were best fitted by the Weibull model and were estimated  $\delta$  values (time to first decimal reduction, equivalent to the D value of linear modeling) of 0.75 min for 110°C ( $R^2 = 0.98$ ) and 24.60 s for 120°C ( $R^2 = 0.97$ ), with shape parameter (p) of 1.39 and 2.64, respectively, indicating a downward concavity in the survivor curve. The z-value was  $30.45^\circ\text{C}$ , suggesting the higher thermal resistance compared to the other beverages.

These findings emphasize the importance of pH in modulating bacterial resistance, with less acidic beverages requiring higher thermal input for effective microbial control. The results aid in optimizing thermal processing parameters for oat-based beverages while maintaining product quality. The study demonstrates the applicability of predictive microbiology in designing safe and efficient pasteurization processes, ensuring food safety in minimally processed plant-based beverages.

**Keywords :** Predictive modeling, thermal resistance, oat-based beverages, *Geobacillus* spp., food safety, non-linear models.

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## OP30

### Longitudinal analysis of microbial diversity and dynamics during storage of chicken products – towards early warning of risks posed by foodborne pathogens.

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Culture-free, direct analysis of the food microbiome by amplicon sequencing or shotgun metagenomics, could offer a more timely assessment of all relevant species present in/on a matrix. Furthermore, longitudinal analysis of food-related microbiomes allows for the detection of meaningful interactions between key microbial species, some of which can function as early warning biomarkers for microbial hazards and risks on food and in the food production chain.

We have studied the diversity and dynamics of bacterial food microbiota of twenty-four chicken products representing four levels of an animal welfare quality mark (zero to three stars). The microbiota of the chicken products were analyzed at three different time points: day of purchase (t1), after three days (t2) and after seven days (t3) of storage at four degrees Celsius. In addition, we determined the presence of *Listeria monocytogenes*, a foodborne pathogen by using classical microbiological methods and whole genome sequencing of isolates. We analyzed the data by using a suite of different clustering algorithms and machine learning techniques (e.g. Random Forest).

The general trend for all products was a drastic drop in the alpha diversity of the bacterial community between t1 and t2 followed by a moderate decrease or slight increase between t2 and t3. On average a higher alpha diversity was observed at t1 for the three star products compared to the one or zero star products, but this difference was no longer significant (t2) or present (t3) at the later timepoints. Spoilage organisms such as *Brochotrix*, *Carnobacterium*, *Leuconostoc*, *Serratia* and *Pseudomonas* were most often found to become the dominant genera after time, although we observed some distinct associations between the animal welfare quality mark and specific genera. The *Listeria* status (+/-) was also a distinctive factor in clustering patterns and showed significant associations with the quality mark. Finally, we observed several associations within and between genera/orders that potentially indicate synergistic and/or antagonistic interactions.

These results indicate the importance of monitoring the microbiome of food-related matrices and the potential to extract meaningful data from these analyses, ultimately contributing to the forecasting of microbial hazards in the food production chain

## OP31

### Dynamic modelling of *Photobacterium iliopiscarium* and *Photobacterium phosphoreum* growth in a modified atmosphere packaging seafood-food model as a function of dissolved gases and pH

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*Photobacterium phosphoreum* is a well-known specific spoilage organism (SSO) in cold-water marine seafood, exhibiting notable CO<sub>2</sub>-tolerance. While research has focused on the influence of headspace gas composition on its growth, no studies have specifically examined the effect of dissolved CO<sub>2</sub>. Additionally, *Photobacterium iliopiscarium*'s CO<sub>2</sub>-tolerance, to the authors knowledge, has never been studied. The present work aims to model *Photobacterium* growth as a function of dissolved gases and pH. Sterile fish pate was used as seafood model, inoculated with either *P. phosphoreum* or *P. iliopiscarium*. Five packaging atmospheres (CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub>) were selected: 0:80:20, 17:53:20, 35:45:20, 53:17:20, and 70:10:20. The headspace gas composition, pH, dissolved CO<sub>2</sub> levels, and *Photobacterium* counts were monitored. The dissolved CO<sub>2</sub> concentration was estimated by tracking changes in headspace gas volume during storage, as CO<sub>2</sub> dissolution in the product's aqueous phase led to package shrinkage. This effect was quantified by immersing the inoculated samples underwater and measuring buoyancy force using a texture analyzer. *Photobacterium* growth kinetic parameters were estimated based on gas diffusivity estimations

and a scaled sensitivity coefficient (SSC) approach. The kinetics were described by using the Baranyi and Roberts model and a gamma concept approach that relates the growth rate with the parameters of interest. SSC showed that only CO<sub>2</sub>max-diss,  $\mu_{\max}$  and pH<sub>1/2</sub> (the pH at which  $\mu_{\max}$  is halved) were large enough for estimation. Regardless of the inoculated species, increasing the initial CO<sub>2</sub> concentration in the headspace resulted in a corresponding rise in CO<sub>2</sub>max-diss in the fish pate, ranging from 55 ppm at the lowest CO<sub>2</sub>-level to 8968 ppm at the highest. In both species,  $\mu_{\max}$  exhibited a similarly strong negative correlation with CO<sub>2</sub>max-diss, showing a comparative decline (from 11.7 to 1.9 l/h) as dissolved CO<sub>2</sub> levels increased within the same range. Notably, when the CO<sub>2</sub>max-diss was at its lowest level of 55 ppm, the growth rate was estimated at 5.2 (l/d). Additionally, pH<sub>1/2</sub> was determined to be 5.06. The findings highlight comparable CO<sub>2</sub>-tolerance between species and emphasize the importance of integrating modelling approaches that incorporate dissolved gases and pH into spoilage risk assessment and packaging design to enhance seafood preservation and shelf-life prediction.

Keywords : *Photobacterium*; Dissolved CO<sub>2</sub>; pH; Modelling; Growth kinetics; Seafood spoilage

OP32

## Modelling The Effects of Food-Intrinsic Characteristics on the Growth Kinetics of Escherichia Coli

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Most predictive models for bacterial growth have been developed on the basis of experimental data obtained from culture media. However, these models often yield different predictions from observations in foods. Although this discrepancy would be due to differences in compositional characteristics, food structure, and other factors, the impacts on bacterial growth kinetics have not been fully quantified. This study aimed to quantify the effect of protein, one of the major food-intrinsic characteristics, on the growth kinetics of *Escherichia coli*. A predictive model incorporating this effect was subsequently developed to bridge the gap between predictions by a culture media-based model and observations in foods. The growth kinetics of *E. coli* ATCC 25922 were investigated at 37 °C in protein mixture comprising albumin (0.001–30% (w/w)) and phosphate-buffered saline. The observed growth kinetics data were analyzed by Baranyi and Roberts model to estimate maximum specific growth rate ( $\mu_{\max}$ ) and maximum population density ( $N_{\max}$ ). These estimated parameters were successfully described as equations of the amino group concentration in the form of the Monod's model

and logarithm, respectively. A predictive model was subsequently developed by incorporating  $\mu_{\max}$  equation into the square-root type  $\mu_{\max}$  model developed by Ross (2003). The model performance was validated by experimentally obtained growth data of *E. coli* in actual foods. The root mean squared error (RMSE) of the developed  $\mu_{\max}$  model was improved (RMSE = 0.225) than that of the model without the amino group concentration (RMSE = 0.242). The changes in bacterial numbers over time were also predicted, and the model with the amino group concentration (RMSE = 0.652) showed an increased prediction accuracy in terms of the RMSE compared with the model without the amino group concentration (RMSE = 0.681). In particular, predictions for lettuce by the model with the amino group concentration (RMSE = 0.661) were substantially improved compared with the model without the amino group concentration (RMSE = 1.015). The developed model incorporating the effect of the food-intrinsic characteristics indicated the potential to explain the discrepancy, which could not be explained by general environmental parameters.

## OP37

### Viable foodborne pathogenic bacteria quantification using PMA-ddPCR assay for quantitative microbiological risk assessment

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Foodborne pathogens are the leading cause of foodborne illness worldwide, representing a significant public health concern that seriously threatens people health and hinders global economic. Quantitative detection of viable foodborne pathogenic bacteria provides a scientific basis for implementing microbiological criteria and conducting quantitative microbiological risk assessments in food. Although the plate count method and the most probable number (MPN) method are widely used to quantify foodborne pathogenic bacteria in food, validated molecular methods are becoming a suitable alternative because of their absolute quantification, rapidity, specificity, and sensitivity. Here, a droplet digital polymerase chain reaction (ddPCR) assay in conjunction with propidium monoazide (PMA) for identifying and quantifying of *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, and

*Cronobacter* spp. using target genes have been established. The PMA-ddPCR method exhibited high specificity and sensitivity with a limit of detection (LOD) of 101 ~ 102 CFU/μL and a limit of quantification (LOQ) of 102 CFU/mL. Moreover, the LOD values obtained using PMA-ddPCR showed higher consistency than those obtained using the plate count method ( $R^2 > 0.99$ ). Furthermore, mathematical modelling of growth kinetics confirmed that the measurement using the plate count method and PMA-ddPCR assay were highly correlated, with a Pearson correlation of nearly ~0.996 and a calculated bias factor value of 0.88. Thus, ddPCR is an acceptable alternative to quantify foodborne pathogens and improves its absolute quantification. The PMA-ddPCR assay is a potentially powerful tool for collecting scientific data to assess food microbiological risks and improve national food safety.

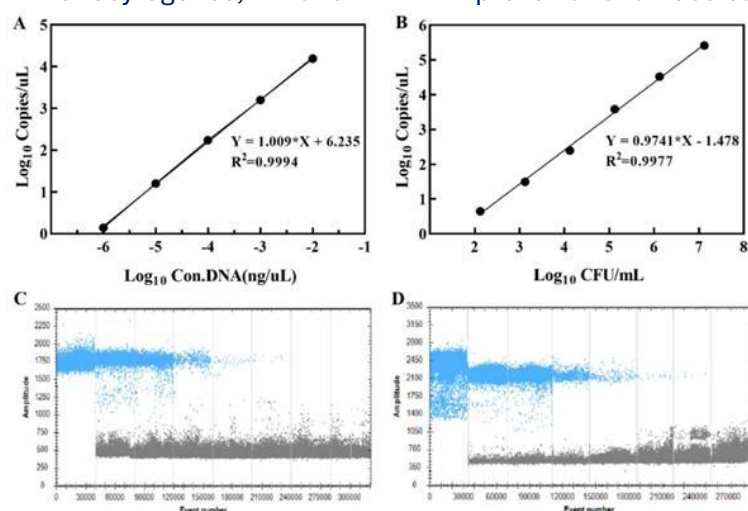


Figure 1 Sensitivity evaluation of the ddPCR assay in the detection of *invA* of *Salmonella* spp.

## OP39

### Modeling *Bacillus subtilis* Sporulation under dynamic pH and Assessing the Spore Properties for Food Safety and Quality Management

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Bacterial spores contribute to food spoilage due to their extreme resistance, making their control a major challenge in food safety. While pH influences sporulation efficiency and spore properties, most predictive models rely on static conditions, limiting their application to dynamic food environments. This study integrates a growth-sporulation modelling approach to quantify and predict *Bacillus subtilis* sporulation and spore properties under fluctuating pH conditions, providing insights into microbial adaptation in food processing.

*Bacillus subtilis* BSB1 was cultivated in 3L bioreactors at pH 7.0 for 16h, followed by shifts to pH 4.0 or pH 5.5 for 134h. A static pH 7.0 condition served as a reference. Growth kinetics were monitored using CFU counting, while sporulation was assessed after heat treatment at 80°C for 10 minutes. A growth-sporulation model was developed to predict sporulation dynamics, integrating cell growth, sporulation initiation, and spore accumulation under pH fluctuations. Spore heat resistance was evaluated through D-value and Z-value determination at different temperatures. Spore germination efficiency was assessed by monitoring germination kinetics using flow cytometry in L-alanine suspension and BHI medium. Expression of genes related to

sporulation, heat resistance, germination, and acid stress was analysed using RT-qPCR to confirm model hypotheses and assess phenotypic variations induced by pH conditions. Results showed a striking correlation between gene expression, sporulation kinetics, and spore properties. At pH 4.0, sporulation was completely inhibited, with key sporulation genes expressed below the quantification limits. At pH 5.5, sporulation was delayed, correlating with prolonged sporulation gene expression. High gene expression variability was observed at pH 7.0, while at pH 5.5, fluctuations were reduced, suggesting fewer metabolic options. Spores formed at pH 5.5 had comparable D-values to those formed at pH 7.0, but with a significantly higher Z-value. Additionally, spores from pH 5.5 exhibited higher germination efficiency, linked to the upregulation of *gerAA* and *gerPC*.

These findings demonstrate how pH fluctuations regulate sporulation through transcriptional shifts and kinetic delays, leading to changes in spore properties. Understanding how dynamic pH modulates spore resistance and germination can help optimize food processing strategies, identify pH thresholds and durations that limit sporulation, improve sterilization efficiency, and reduce spoilage risks.

## OP46

### Quantitative approaches to evaluate the growth rate and acidification capacity of Lactic acid bacteria (LAB) isolated from sheep cheese

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Predictive models are often used to quantify LAB and pathogen behaviours in food systems. Although LAB kinetics in artificial media are well studied, their acidification patterns in sheep milk and milk serum remain unexplored. This study aimed to evaluate LAB growth and acidification capacity at 30 °C and 37 °C in both artificial media and sheep milk serum to better understand LAB behaviours in cheese environments.

LAB growth kinetics were evaluated by measuring OD600 in a microplate reader after serial two-fold dilutions in sheep milk (SM), sheep milk serum (SMS), MRS, GM17, and LM17 media at 30 °C and 37 °C. Growth rates ( $\mu_{max}$ ) were derived from optical density time-course data. Acidification dynamics were tracked via fluorescence using 5-(6)-carboxyfluorescein (1  $\mu$ M), with pH recorded. Fluorescence signals were smoothed using a Savitzky-Golay filter, and acidification capacity was determined by integrating the curve from initial pH to pH 4.8. The area was computed via the trapezoidal rule and normalised to growth rate ( $\mu_{max}$ ) to derive an acidification efficiency ratio.

LAB isolates exhibited variable growth and acidification patterns influenced by strain,

medium, and temperature. *Lactiplantibacillus plantarum* S7 and S8 demonstrated the most efficient acidification in sheep milk and SMS at 30 °C, with moderate growth rates of 0.57 and 0.64, respectively. In contrast, *Levilactobacillus brevis* 5.17 exhibited a high growth rate of 1.39 but a low acidification curve area of 322.2, indicating a decoupling of biomass accumulation and acid production. In GM17 medium, *Limosilactobacillus fermentum* 5.10 and *Companilactobacillus halodurans* I2.8 were the most effective acidifiers at 37 °C, with acidification-to-growth ratios of 67.3 and 51.9, respectively. Similar results were observed in LM17, where I2.8 grew well (1.314) but was surpassed by 5.10 in acidification efficiency at 30 °C. At 37 °C, *L. casei* 3.13 was the most effective strain in terms of acidification relative to growth. In MRS medium, S2 and 5.17 showed high growth (0.97 and 0.99) but low acidification, whereas S7 stood out with the highest acidification-to-growth ratio of 90.3 at 30 °C. These results reinforce the need for careful strain and condition selection when designing LAB-based fermentations for dairy products

**Keywords :** Predictive modelling; Lactic acid bacteria; Growth rate; Acidification; Cheese

## OP47

### Synergistic Effect of Lactic Acid Bacteria and Initial Ph of a Milk Model on the Control of *Listeria Monocytogenes* During Fermentation

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Previous research showed that strains of *Leuconostoc mesenteroides*, *Lactocaseibacillus paracasei* and *Loigolactobacillus coryniformis* isolated from goat's raw milk cheeses — presented promising antilisterial activity and acidification properties. The objective of the study was twofold: (1) to determine whether the initial pH of a milk model (as a fermenting substrate to produce cheese) affects the growth kinetics of *Listeria monocytogenes*, in monoculture and coculture with each of the lactic acid bacteria (LAB) strains; and (2) to quantify and compare the capacity of the LAB strains to inhibit *L. monocytogenes* in the same milk model substrate. Heat-treated reconstituted milk (HTRM) was used as milk model in the experiments. Challenge tests were conducted in HTRM adjusted to three initial pH levels of 5.5, 6.0, and 6.5, inoculating *L. monocytogenes* in monoculture, and each of the LAB strains in monoculture and in coculture with the pathogen. Milk samples were incubated at 12°C during 8 days. A pH-driven dynamic growth model was fitted to all experimental growth curves. In monoculture, *L. mesenteroides* consistently exhibited the highest growth rates

and maximum concentrations in milk at all initial pH levels. In coculture, this strain more effectively controlled *L. monocytogenes* by reducing its growth rates (day<sup>-1</sup>) from  $3.201 \pm 0.045$ ,  $3.416 \pm 0.177$ , and  $3.432 \pm 0.073$  at initial pHs 5.5, 6.0 and 6.5, to  $1.469 \pm 0.205$ ,  $2.293 \pm 0.284$ , and  $1.552 \pm 0.132$ , respectively. Furthermore, as the initial milk pH increased, the maximum concentration of *L. monocytogenes* in monoculture and in coculture also increased, although the three LAB strains were able to reduce the maximum load of *L. monocytogenes* at all pH values ( $p < 0.01$ ). These findings indicate that, among the three tested LAB strains, *L. mesenteroides* is the most efficient in slowing down the growth and decreasing the maximum load of *L. monocytogenes* in fermenting milk, although for all LAB strains, it was demonstrated that the initial pH of milk has an effect on the extent of inhibition of *L. monocytogenes*. Thus, adjusting milk to a more acidic pH before fermentation has a synergistic effect with the addition of LAB on the control of *L. monocytogenes*.

## OP55

### Non-invasive spoilage prediction of aerobically stored sea bream: A comparative study of machine learning models using multispectral imaging for real-time quality assessment

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**Introduction:** Microbial spoilage is a challenge to the fish industry, affecting food safety, shelf-life, and consumer health. Real-time, non-invasive spoilage monitoring is essential to minimize waste and improve quality control. Spectroscopic sensors, combined with machine learning (ML), offer a promising approach for automating spoilage prediction. However, ML performance depends on dataset characteristics, and no single model is universally optimal. This study explores how different ML models influence predictive accuracy, emphasizing the need for customized model selection in food science.

**Methodology:** Multispectral imaging (MSI) data from two sensors, benchtop-MSI (n=195) and portable-MSI (n=198), were used to assess spoilage in sea bream fillets stored at 0, 4, 8 and 12 °C, under aerobic conditions. Spectral data were combined with microbial spoilage indicators, including total viable count (TVC), heterotrophic marine bacteria, H<sub>2</sub>S-producing bacteria and *Pseudomonas* spp. populations. Stratified sampling was applied where 80% of the total dataset was used for training and 20% for testing the models. A total of nine ML regression models were tested with different spectral preprocessing techniques and cross-validation strategies, to optimize performance. Model evaluation was based on Root Mean Squared

Error (RMSE), R-squared (R<sup>2</sup>), and prediction accuracy, to determine the most effective approach for each case.

**Results:** Model performance varied and no single model outperformed others, across all cases. For benchtop-MSI, the Support Vector Machine (linear kernel) yielded adequate performance for TVC (R<sup>2</sup>=0.71, RMSE=0.80, prediction accuracy=75.68%), whereas Partial Least Squares performed best for H<sub>2</sub>S populations (R<sup>2</sup>=0.50, RMSE=1.01, prediction accuracy=55.56%). Portable-MSI demonstrated overall lower predictive power, compared to benchtop-MSI, with the Ridge model having the best results for *Pseudomonas* spp. (R<sup>2</sup>=0.45, RMSE=1.08, prediction accuracy=55.26%).

**Conclusions:** The findings of this work reinforced that model selection must be adapted to the specific dataset and parameter, as a one-size-fits-all approach is ineffective. The importance of a data-driven, dynamic approach to ML model selection in food quality assessment highlights the need for adaptive strategies, rather than relying on a single algorithm. These insights contribute to the development of real-time, non-invasive tools for food quality monitoring, offering potential applications across various food commodities to enhance food safety and reduce waste in the food industry.

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## OP56

### A probability-based growth/non-growth boundary model for bacterial populations at single-cell level

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**Introduction:** Growth probability near the growth/non-growth boundary depends on initial inoculum size, yet a theoretical model from the single-cell perspective is lacking.

**Purpose:** We developed two types of mathematical models to estimate the probability of individual bacterial cell growth at various initial inoculum levels and considered which model describing better.

**Methods:** *Escherichia coli* and *Bacillus cereus* were each inoculated into tryptic soy broth supplemented with glycine, sodium chloride, or ethanol in triplicate 96-well microplate experiments. Six initial inoculum levels (0, 1, 2, 3, 4, 5 log CFU/well) were tested at 25°C for up to 7 days. Turbidity was recorded before incubation and on days 3 and 7; wells showing an increase in optical density greater than 0.1 were defined as positive for growth. Growth probability under each condition was the proportion of positive wells. We developed two types of models; a single-hit model and exponential model, which

are known as dose-response models. A Bayesian approach was used to fit each model, yielding estimates of individual cell growth probability that scale with initial inoculum size. After estimating the parameters, we compared the value for accuracy of each model.

**Results:** In all test conditions, higher initial inoculum levels corresponded to higher growth probabilities. The single-hit model captured these trends effectively, with root mean squared error (RMSE) below 0.40 on day 3 and below 0.50 on day 7, indicating robust predictive performance across both species and stressors.

**Significance:** These simple yet interpretable models generalize the growth probability at any starting cell count from an estimated single-cell growth probability. Incorporating these approaches into existing boundary models will strengthen risk assessments and food safety predictions by accounting for the influence of initial bacterial load.

**Keywords :** predictive microbiology, inoculum size effect, single-hit model, food preservation

## Part D – Back to Future Roots of PMF

### OP05

## Data-Driven Tools for Optimizing Microbiological Monitoring in Dairy Production with a Risk-Based Approach

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Microbiological monitoring of milk and dairy products, such as raw milk cheeses and milk powders, relies on a deep understanding of manufacturing processes and associated microbiological hazards. Pathogenic bacteria originating from bovine fecal excretion or sporadic environmental contamination, along with hygiene indicators, are monitored at various stages of production: raw milk, blended milk, intermediate products, finished products, and the production environment.

To optimize microbiological surveillance in terms of consumer risk, cost, and efficiency, a proactive, data-driven approach is essential, based on routine results and mathematical modeling, as recommended by international organizations such as ICMFH, JEMRA, and national food safety agencies.

In recent years, the French dairy sector has developed decision-support tools to optimize microbiological monitoring based on self-monitoring results and advanced statistical methods.

These tools include:

- Models to assess the temporal evolution of contamination data (farm milk, products, environment) and the effectiveness of control measures (e.g., milk sorting) using statistical tests. This is particularly challenging when milk from several farms is mixed.

- Models to evaluate the correlation between the prevalence of hygiene indicators above a certain threshold and pathogen prevalence, helping to identify relevant hygiene indicators for the HACCP plan.

- R-CHART for quantitative data (e.g., E. coli counts), P-CHART for dichotomous data (presence/absence), F-CHART for rare event detection, and moving average analysis. These tools provide a dynamic view of analytical results, enabling early detection of weak signals and proactive implementation of reinforced control plans before contaminated batches reach the market.

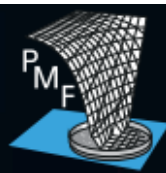
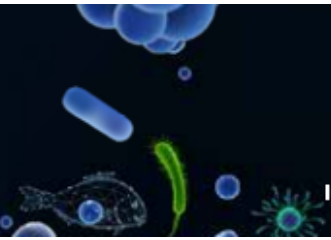
- A sampling plan efficiency calculator for infant milk powder and raw milk cheeses, evaluating detection performance based on historical analytical data and contamination level distributions. When quantitative risk assessment models are available (especially for raw milk cheeses), the risk reduction percentage for a given sampling plan is also calculated, allowing for a calculation of its added value in terms of risk and cost.

These tools integrate quantitative approaches based on mathematical modeling, requiring structured, high-quality data organization. To facilitate implementation, standardized data entry templates are provided to dairy professionals, and the tools are available through interactive web applications, helping stakeholders strengthen monitoring and process control while optimizing analytical resources.

**Keywords :** Microbiological monitoring, dairy industry, quantitative modeling, control charts, sampling plans, risk-based approach

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## OP06

### Estimation of Kinetic Parameters During Microbial Growth Under Dynamic Temperature Conditions

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In recent literature for microbial growth in foods, there are limited studies that evaluate different dynamic temperature profiles in terms of their ability to estimate kinetic parameters. Most studies focus on specific periodically repeated temperature patterns, such as those including two or three isothermal steps, forming box or chair shapes, respectively. However, the selection of the particular profile can significantly affect the accuracy of the estimated kinetic parameters. Furthermore, in kinetic studies of microbial growth, mean values of primary and secondary model parameters are often presented without their corresponding confidence intervals. Even in cases where parameter uncertainty is reported, the calculation methodology is rarely specified. This is important since in many cases uncertainty simply represents the standard deviation of replications of raw data, which can underestimate the true uncertainty in parameter estimation. The case of microbial growth under dynamic conditions is governed by differential

equations and the methodology for estimating confidence intervals is non-trivial, making its reporting and analysis critical. In the present work, a comparison between various models of microbial growth was performed in terms of their ability to describe experimental data, including also the estimation of the uncertainty of the kinetic parameters through asymptotic confidence intervals. In silico data were generated to assess the effect of different temperature profiles on the accuracy of estimating kinetic parameters. Temperature profiles that lead to sigmoidal shaped growth curves did not allow for accurate parameter determination. Therefore, modeling microbial growth under dynamic temperature conditions requires special attention to the selection of temperature profile. Estimation of kinetic parameters with high uncertainty can significantly affect the reliability of microbial growth predictions, emphasizing the need for rigorous experimental and computational approaches.

**Key words:** Confidence intervals, primary and secondary model parameters, dynamic modeling

## OP07

### Evaluation of Heat-Treated Lactic Acid Bacteria for Postbiotic Production in Food Biopreservation

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Postbiotics, defined as bioactive compounds produced by probiotic bacteria, have attracted growing interest due to their potential health benefits and superior stability compared to live probiotics. Their resilience under various conditions makes them promising candidates for practical applications, particularly in food biopreservation.

This study investigated the effect of different thermal treatments on the production of postbiotics from lactic acid bacteria (LAB) strains, aiming to identify optimal conditions for enhancing antimicrobial activity while ensuring microbial inactivation. The LAB strains used included *Lactiplantibacillus plantarum* F2, 40 (*Pediococcus acidilactici*), 50 (*Pediococcus pentosaceus*), and AF1 and AF2 (both *Leuconostoc mesenteroides*). Each strain was subjected to heating at 60 °C and 80 °C for 30 minutes, and at 121 °C for 15 minutes. Following treatment, samples were assessed for cell viability and the antimicrobial efficacy of the resulting postbiotics.

Antimicrobial activity was tested against four foodborne pathogens: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 43300, *Salmonella enteritidis* ATCC 13076, and *Listeria monocytogenes* ATCC 7644. Results showed

that cell viability varied according to both bacterial strain and treatment temperature. At 60 °C, strains AF1 and 40 retained viability, indicating incomplete inactivation; therefore, their postbiotics were excluded from further antimicrobial assays. In contrast, treatments at 80 °C and 121 °C successfully inactivated most strains, yielding postbiotics with differing levels of antimicrobial activity.

Postbiotics derived from strain 40 at 80 °C exhibited strong inhibition against three of the four tested pathogens. Strain 50 showed the most consistent and potent antimicrobial activity at 80 °C across all pathogens. For strains AF1 and AF2, the antimicrobial effects varied depending on the pathogen, while strain F2 demonstrated the highest activity following treatment at 121 °C.

In conclusion, the study demonstrates that thermal treatment plays a crucial role in determining both the safety and efficacy of LAB-derived postbiotics. Heating at 80 °C emerged as a generally effective condition, although optimal results varied among strains. These findings support the development of strain-specific protocols for producing functional postbiotics suitable for food biopreservation.

**Keywords :** Lactic acid bacteria, postbiotic, food safety, food biopreservation

## OP15

### A Decision Support Tool for Real time monitoring and Forecasting Fungal Growth in Grain Silos Based on Sensor Data and Climate Models

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Effective control of fungal growth in grain storage is critical for ensuring food safety and quality, necessitating innovative tools for proactive risk management. This study presents a novel R Shiny application tool designed to predict real-time fungal growth in grain silos using environmental data, specifically temperature and dew point temperature, collected from meteorological sensors. The application incorporates short-term predictions based on weather forecasts, enabling proactive management strategies to mitigate fungal risks. Additionally, it integrates long-term climate projections focusing on the impacts of climate change on fungal growth patterns, providing a comprehensive understanding of future fungal risks and enterprise sustainability.

The application uses sensor data (temperature and dew point temperature) to estimate relative humidity using the August-Roche-Magnus approximation on an hourly basis, which in turn is applied to a developed model to calculate the expected equilibrium moisture content of corn as a function of temperature and relative

humidity. Subsequently, the grain's water activity is computed using its sorption isotherm via the Guggenheim-Anderson-de Boer (GAB) model. These estimated water activity values, along with sensor temperature data, serve as inputs for a series of secondary predictive models for fungal growth (*Aspergillus*, *Fusarium*, *Penicillium* etc.) collected from literature to determine lag time and growth rate. The primary growth model then forecasts fungal growth in real-time, allowing stakeholders to assess risk dynamically and make timely interventions based on expected weather forecasts for the upcoming days.

This approach provides a novel tool for integrating meteorological data into predictive microbiology, aiding in decision-making processes for grain storage management and fungal risk reduction.

This study highlights the potential of real-time and forecasting modelling applications in food safety and storage management, offering a practical tool for industry professionals to mitigate fungal risks based on site-specific weather conditions and climate change.

**Acknowledgement:** The research work has received funding from the European Union's Horizon Europe Research and Innovation Programme under the HORIZON-CL6-2024-FARM2FORK-01 call project AMBROSIA "Bridging Knowledge, Communication, and Action for Food Safety in a Changing Climate" (Grant Agreement: 101181300).



## OP16

### The recent work from JEMRA and FAO on microbiological risk assessment

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Since the 2023 ICPMF, the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) and FAO have continued their work on microbiologically-related food safety issues.

- JEMRA meeting on viruses in foods. Part 1: food attribution, analytical methods and indicators
- JEMRA meeting on viruses in foods. Part 2: prevention and intervention measures
- FAO and WHO workshop on microbiological risk assessment in South African
- FAO workshop on risk ranking in Mongolia, Vietnam, Tanzania and Uganda
- FAO workshop on microbiological risk assessment for the Codex member countries
- FAO expert meeting on Clostridium in foods
- FAO workshop in water safety used in foods in Honduras
- FAO expert meeting on parasites in foods

This presentation will provide an in-depth look at the application, experiences, and ongoing developments of microbiological risk assessment (MRA) at the international level, with a particular focus on the unique needs and challenges faced by low- and middle-income countries. It will also cover the emerging global issues that are shaping the future of food safety, as well as the FAO's plans and initiatives to

address these challenges. It will emphasize how science and evidence-based approaches can be leveraged to inform decision-making, strengthen agrifood safety systems, and promote the harmonization of international food trade practices.

From the FAO's perspective, there is significant value in collecting and harmonizing information, frameworks, and tools related to international MRA. This harmonization allows for a more comprehensive understanding of food safety risks on a global scale, identifying similarities and commonalities between regions and countries. It also provides a critical platform for addressing global food safety issues that transcend national borders, helping to develop cohesive solutions for shared concerns. Furthermore, MRA offers the opportunity to assess the availability and quality of data on a global scale, pinpointing areas where knowledge gaps exist and where further research or data collection is needed.

By fostering collaboration, aligning global standards, and ensuring that the scientific basis for food safety decisions is robust and universally applicable, MRA can play a key role in enhancing public health protection and facilitating safer, more equitable international food trade.

## OP17

### Smart fermentation with digital twins: a support decision tool for managing, optimising quality and energy performance, applied to the fermentation of plant-based products.

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The use of digital twins at the industrial scale is difficult due to complexity of model aggregation, spatial and temporal heterogeneity of processes, computing time, resistance to change. This study is dedicated to deployment of a digital twin of the plant base product fermentation and aims to develop a generic methodology of the deployment of digital twins in the agri-food sector and demonstrate the great possibilities offered by these tools. The prototype development is based on three steps: i) Expert knowledge modelling; ii) Services; iii) Digital twin instantiation.

**Expert knowledge modelling:** Our process is based on the fermentation of plant based raw material in a bioreactor. The suggested model encompassed the impact of the substrate, temperature and acidity (pH and acid concentration) on the starter growth, and on pathogens or spoiling spore forming bacteria (germination, growth and sporulation). The bioreactor use requires careful consideration of the hydraulic and thermal phenomena during the fermentation phase.

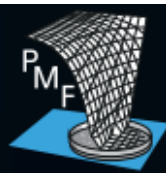
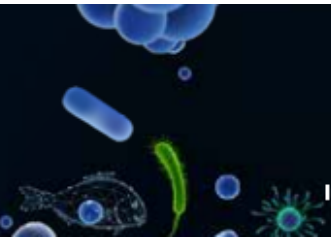
**Services:** Real-time monitoring provides the operator with a global view of the fermentation parameters, enabling changes to be made during the process. Analysis of the correlation between the different variables and the fermenter quality indicators were suggested. Prediction of reactor behaviour offers the operator the possibility of changing fermentation conditions and suggests corrections. Simulation can be carried out independently of the physical fermenter, allowing different hypotheses to be explored.

**Digital twin instantiation:** The ontology of bioreactor equipment has been investigated to ensure formalised collaboration for the various stakeholders and interoperable models. This conceptual level defined above must be connected bi-directionally with the physical system (hardware and software) and its various representations in order to form this consistent digital image, and federating the different models to target the use cases. This step represents the culmination of the conceptualisation, formalisation and technological adaptation aspects.

**Keywords :** starters, spoilage, plant based product, Bacillus, real time

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## OP18

### A Framework for Assessing Microbial Risks Related to Climate Change in Food Safety

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Climate change poses significant challenges to food safety, particularly regarding microbiological risks influenced by variable environmental conditions. Increasing atmospheric temperatures, altered precipitation patterns, and extreme weather events impact pathogen survival, transmission, and contamination routes, heightening uncertainties in microbial risk assessment. To address these challenges, a systematic, data-driven approach is essential for evaluating and mitigating risks associated with climate change.

The proposed framework is presented in the form of a “White Paper”, assessing the impact of climate change on microbial food safety by integrating climate change projections, predictive microbiology, and Quantitative Microbial Risk Assessment (QMRA). The framework establishes links between climatic variables and microbial behavior, identifying key factors that influence pathogen prevalence and food contamination risks. To bridge this problem, the framework integrates future climate scenarios from regional climate models with the development of impact models that quantify microbial risks under different climate scenarios. The impact models will be constructed using machine learning techniques, particularly neural

networks, to analyze complex interactions between climatic variables and microbial behavior. Predictive microbiology is utilized to simulate pathogen survival, growth, and inactivation in response to environmental conditions such as temperature, water activity, and pH. QMRA is integrated to estimate foodborne risk by combining exposure assessment, dose-response modeling, and contamination pathways. Finally, given the inherent uncertainties in both climate models and QMRA, the proposed framework incorporates systematic uncertainty analysis following the EFSA guidelines. Thus, reliability and interpretability of the assessment is improved, without propagating the uncertainty between the two modeling systems. Addressing the challenges of climate-driven microbial risks requires multidisciplinary collaboration and international data-sharing efforts to strengthen model accuracy and applicability. By combining climate science, microbiology, and quantitative risk assessment, this framework provides a scalable tool for policymakers, regulatory bodies, and the food industry to anticipate and mitigate climate-induced risks, ensuring a proactive approach to food safety in a changing global environment.

This research was part of the project AMBROSIA under funding from the European Union’s Horizon research and innovation program under grant agreement No101181300.

## OP19

### Modelling the growth and growth boundaries of *Listeria monocytogenes*: focus on strain variability and organic acids

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**Introduction:** There is a need for the food industry and the inspection authorities to have at their disposal models that accurately predict the combined effect of product characteristics and storage conditions on the ability of *Listeria monocytogenes* to grow. The aim of this study is to develop a new stochastic model for the growth and growth/ no growth of *L. monocytogenes*, including both strain variability and the inhibitory effects of organic acids (lactic, acetic, propionic, citric and sorbic).

**Material and Methods:** The growth and growth limits of *L. monocytogenes* is based on a cardinal model with 'interaction term' that describes the interactive effects of temperature, pH, aw, concentrations of undissociated acid(s) in water. A literature search was conducted to define the distributions for the strain-dependent model parameters. Growth boundaries were predicted by the means of 1D Monte Carlo simulations. Predictions of growth/ no growth were assessed on a validation data set of 3054 literature data, including 1617 in the presence of organic acid(s). The criteria were the correct prediction percentage (CPP), the positive predictive value

(PPV), and), the negative predictive value (NPV). The growth model was also validated on literature data in dairy products (30 challenge tests).

**Results:** The distributions for the MICs of lactic, acetic, citric and propionic acids are based on individual values reported for 33, 15, 8 and 10 strains, respectively. For sorbic acid, due to the very little information available, the distribution for MIC was based on expert opinion. For the growth/no growth model, the CPP, PPV and NPV values are respectively 96.4, 96.0 and 97.4%. When considering the data in presence of organic acid, the NPV value is 99.0%. Data gaps in the validation data set were identified, where the model should be used with some caution. The growth model was found to provide either accurate or fail-safe predictions of the kinetics of *L. monocytogenes* in cheese.

**Conclusion and significance:** This model can support the food industry in formulating food products that are safe by design and access food processes with regard to the risk of *L. monocytogenes*.

## OP28

### ZooNotify – An interactive data tool for searching and visualizing zoonoses monitoring results along the food chain in Germany

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Availability of data is a major bottleneck in predictive microbiology and quantitative microbial risk assessment (QMRA). Reliable estimates of prevalence, bacterial concentrations and antibiotic resistance are crucial for modelling pathogen transmission along the food chain and conducting consumer exposure assessments.

ZooNotify comprises data from the representative zoonoses monitoring along the food chain and the Salmonella control programmes in poultry flocks in Germany. It makes existing data on the occurrence of zoonotic agents and related antibiotic resistance in the food chain in Germany findable, accessible, interoperable and reusable (FAIR). This data enables risk assessors to track trends over time and across different food production stages, supporting early warning systems and mitigation strategies. The majority of the data is related to livestock and foods of animal origin. However, data is also available on feed, wild animals and plant-based foods, thereby taking a more holistic One-Health approach and including rarely available data.

Currently, ZooNotify contains antibiotic resistance and typing data for 34.956 and 16.619 bacterial isolates from animals and food,

respectively. Additionally, it provides more than 600 prevalence estimates, including confidence intervals, for different zoonotic agents along the food chain. The data is partially aggregated, but we are currently making efforts to provide unaggregated data. Upcoming quantitative data on bacterial concentrations in different foods will allow for dose-response modelling and refined risk calculations.

ZooNotify allows customized data searches based on user interests, with data available for download in a standardized format (.csv). The tool also offers intuitive data visualisations with the option to download generated figures. Each section of ZooNotify is accompanied by clear and concise explanations, ensuring ease of use and comprehensive understanding of the data.

The continuous development of ZooNotify improves the accessibility and reusability of zoonoses data for predictive microbiology and QMRA. It further aligns with the EU strategy for open data and forms an important basis for the protection of animal and consumer health. Although data on ZooNotify is only available for Germany, it can still be useful for the modelling community, where reliable data sources are often scarce and best available estimates must be used for risk assessments.

Keywords : database, dashboard, prevalence data, antimicrobial resistance, concentration data

## OP40

### A Mathematical Model to Predict the Effect of Temperature and Water Activity on the Growth of *Alternaria* Spp. in Oats

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Climate change represents a global challenge to food safety, particularly concerning *Alternaria* spp., a mycotoxigenic fungus. Oats are among the cereals with the highest incidence of *Alternaria* contamination due to their open-field cultivation and the favourable environmental conditions. This study assessed and modelled the growth of four *Alternaria* isolates from oat grains cultivated in the Ebro Valley (Spain) as a function of temperature and water activity (aw). The isolates were identified through ITS and TEF sequencing as *Alternaria alternata* (three isolates) and *Alternaria arborescens* (one isolate). *Alternaria* isolates were inoculated (ca. 100 spores) onto the surface of irradiated oat grains. The strains were incubated at temperatures ranging from 5 to 45 °C and at aw values of 0.90, 0.94, and 0.98 for 30 days, with growth periodically assessed by measuring the colony radii. The Baranyi and Roberts model was fitted to estimate the biokinetic growth parameters, while the cardinal parameter models (CPM) were used to determine the minimum, optimum, and maximum temperature

and aw values, as well as the optimum growth rate ( $\mu_{max}$ ) under these specific conditions. All strains grew between 5 and 35 °C, with an optimal aw of 0.98. For *A. alternata*, the  $\mu_{max}$  ranged from 0.69 at 5 °C to 3.99 mm/d at 35 °C, while for *A. arborescens*, the  $\mu_{max}$  ranged from 0.66 to 0.94 mm/d at the same temperatures. The CPM estimated a similar optimal growth temperature for both strains (i.e., 29 °C), with  $\mu_{max}$  of 6.25 mm/d for *A. alternata* and 5.48 mm/d for *A. arborescens*. However, the maximum temperature predicted by the model was higher for *A. alternata* (i.e., 39.9 °C) than for *A. arborescens* (i.e., 35.2 °C), indicating greater heat sensitivity in *A. arborescens*. Neither strain grew above 40 °C, and the minimum growth limit was at aw 0.86 for all strains. These results suggest that *A. alternata* has a greater capacity to adapt to extreme temperatures than *A. arborescens*. Predictive models help identify conditions favouring *Alternaria* toxin accumulation in oats, allowing risk anticipation, control optimization, and enhanced food safety.

**Keywords:** *Alternaria* spp., ecophysiology, thermal adaptation, cardinal parameter model, predictive mycology.

**Acknowledgement:** This work was supported by the Spanish Ministry of Science and Innovation through the project PID2023-148722OB-I00 (funded by MCIN/AEI541/10.13039/501100011033 and FEDER, UE), and the EU through Project 101079173-FunShield4Med (HORIZON-WIDERA-2021-ACCESS-03).

## OP41

### Sensitivity analysis methods for effective decision-making

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<sup>1</sup>Wageningen University, , Netherlands

Quantitative microbial risk assessments (QMRA) aim to estimate the impact of microbiological hazards on public health and to provide a quantitative scientific basis for risk management decisions. Sensitivity analyses are performed to identify key parameters that affect the risk of illness (i.e., Pill), and outputs are the base for the selection of scenarios evaluating the impact of risk mitigation strategies.

The aim of this work was to evaluate the efficacy and robustness of different sensitivity analyses methodologies and to assess to which extend they capture the impact of parameters on Pill. The QMRA of *L. monocytogenes* in RTE cooked meat products (Van der Vossen et al., 2024, 10.1016/j.ijfoodmicro.2023.110516) was used as showcase to compare three sensitivity analyses. First, the Spearman's rank correlation test was used taking into account all billions of simulations of Pill. Second, only the simulations that resulted in an illness case based on Bernoulli trials were used as input for the Spearman's rank correction test. And third, the Sobol sensitivity analysis was used taking into account all simulations. In parallel, scenarios analysis were performed by truncating

parameters to evaluate the parameter's impact on the number of cases.

The Spearman's rank test based on all simulations was not able to successfully capture the impact of all relevant parameters on Pill. Particularly, this was the case for the maximum population density (Nmax), most probably because, despite the variability in the parameter, in most of the simulations Nmax was not reached. When the data set was restricted to simulations that resulted in illness cases, the parameter initial concentration (N0) was not captured as relevant, while the scenario analyses clearly demonstrated that changes in N0 affected the number of cases. On the contrary, the Sobol analyses were able to successfully capture the impact of all relevant parameters due to their ability to measure the effect of interactions between variables.

These analyses highlight the need to use sensitivity analysis approaches that are able to determine the relevance of factors to make the most of the information provided in QMRAs and to identify intervention strategies with the highest impact on risk mitigation.

## OP52

### Tackling One Health risks: How Large Language Models are leveraged for Risk Negotiation and Consensus-building.

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Fighting hunger, ensuring food safety, and promoting health—all while fostering a sustainable, resilient circular economy and protecting ecosystems—are top priorities for policymakers worldwide. To achieve these goals, multi-sectoral approaches and effective risk analyses are needed to mitigate negative impacts on human, animal, plant, and environmental health. However, balancing the costs and benefits for diverse stakeholders often suffers from a lack of transparency and comprehensive methods. In this context, AI technologies, such as Large Language Models (LLMs), offer promising avenues to streamline problem definition, build consensus on risks and priorities, and develop strategies to address these risks. Yet, practical processes to operationalize AI-assisted, participatory risk assessment remain scarce. Our study aims to fill this gap by defining and testing a procedure that leverages state-of-the-art LLMs alongside an agent-based modeling framework for negotiation-centered risk analysis. This proof-of-concept has two main objectives: i) To develop a detailed procedure for applying the framework through a multi-agent modeling process in a Human-in-the-loop (HIL) approach; ii) To evaluate the framework's

applicability via two case scenarios: The spread of infectious agents and biopesticide resistance (scenario 1); targeted wild animal population control (scenario 2). In-field scientists, acting as stakeholders, applied the framework to these real-world One Health challenges. We showcased that our AI-assisted risk negotiation process facilitates active participation from stakeholders across diverse disciplines, enabling them to devise balanced solutions that address various risk dimensions and trade-offs. Integrating LLMs within multi-agent frameworks allows for the partially automated analysis of multidimensional, heterogeneous data, thereby accelerating the consensus-building process. The successful testing of our process demonstrates its potential to support objective, effective risk management in the One Health context. By combining artificial and human intelligence under expert supervision, our approach helps policy and risk managers make balanced decisions under time constraints and amid vast amounts of data. This synergy of AI and human judgment represents a significant advancement over the few existing cross-sectional approaches to risk analysis that incorporate stakeholder engagement.

## OP58

### Application of Predictive Microbiological Models in Industry: A Fit for Purpose Approach for Food Safety Assessment

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Predictive microbiological models are widely used in food industry to assess the safety of food products by design. However, the outcome of these models can vary depending on the system being modeled. This abstract presents a comparative analysis using *Bacillus cereus* as a case study.

The analysis considers three different layers of safety assessment. Broth systems are the first layer representing a cost-effective solution but an oversimplification of the complexity of food systems. The second layer involves models developed in food matrices, which capture the effects of nutrient availability and other rheological behaviors within the food matrix. The third layer incorporates the food microbiota, providing a more realistic representation of the microbial interactions in the food ecosystem.

The perfect outcome will not derive from any system because probably as stated years ago "All models are wrong, but some are useful". Therefore, how can industry navigate through the different outcomes? We present a comprehensive evaluation of *B. cereus* predictions using different models and considering the practical implications for risk assessment.

The results of the comparative analysis will provide insights into the strengths and limitations of each layer from an industry point of view. By identifying a Fit for Purpose approach, the food industry can gain flexibility without compromising food safety. This analysis will contribute towards the advancement of the application of predictive microbiological modeling.

**Keywords :** comparative analysis, food microbiota, food safety, risk assessment, industrial applications

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## Part A – The old good times

### PP02

#### Quantitative Microbial Risk Assessment (QMRA): case study with zoonotic *Anisakis* parasite in fishery products in France in a global market context

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In European countries, consumption of fishery products has been stable in recent years. However, the relative share of aquaculture is increasing, as is the consumption of raw seafood. Finally, fishery products contribute greatly to a balanced diet, but sustainable consumption is necessary to avoid food waste and environmental impact. Proper freezing of “raw” fish before consumption, the most common way to kill parasites, is energy expensive and is not always known and implemented by consumers. The detection of anisakids by consumers can also induce food reluctance.

Two recent EFSA opinions (2024) reassess the presence of zoonotic parasites in fishery products. A systematic review of anisakids occurrence in fishery products was carried out during the publication period 2010-2023 and data were recorded in the “Pathogens in foods” (PIF) database.

The objective of this work was to establish a stochastic QMRA model to predict the number of cases of anisakidosis in relation to the

consumption of fishery products for a population of French adults, in particular for those who purchase fresh fish. The origin, frequency of consumption of raw fish and portion size of the most consumed fish species in France were established from consumption databases and fisheries statistics. The meta-analysis of *Anisakis* data in the PIF database provides estimates of the prevalence and level of contamination of fillets. The effectiveness of home freezing/cooking practices on parasite viability, and the published dose-response relationship were introduced into the QMRA model. Different risk mitigation strategies mainly drawn from the results of the PIF meta-regression analysis were applied to establish different cost-benefit scenarios (*Anisakis* risk vs environmental impact). The source of uncertainties are quantified second order modelling and ranked by sensitivity analysis in order to prioritize data gaps. This QMRA illustrates the challenge of the zoonotic multi-host approach from farm/sea to fork and the potential applications of the PIF database.

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## PP03

### Modeling the Survival of *Salmonella Enterica* in Spaghetti-Like Carrot Strands as Influenced by Temperature and Relative Humidity

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Thinly sliced carrots are consumed around the world as raw ready-to-eat foods. These products may be associated with outbreaks and recalls due to contamination with foodborne pathogens. This study developed mathematical models to predict the survival of *Salmonella enterica* in spaghetti-like strands carrots at different temperature and relative humidity (RH) conditions. Carrots were cut into spaghetti-like strands (2 mm in diameter; 3 g) and inoculated with a cocktail of different *S. enterica* serovars (~7 log CFU/g; *S. Typhimurium* H3380, *S. Enteritidis* ATCC BAA-1045, *S. Newport* J1890 and *S. Saintpaul* FSIS 039). Inoculated samples were placed in desiccators containing saturated calcium nitrate solutions used to create controlled RH environments (~75, 85 and 95%). The desiccators were stored at 6, 9, and 12 °C. After 24 h, *S. enterica* cells were enumerated in samples at time zero (0 h) and every 24 h over 7 days. The survival rates of *S. enterica* in carrots were adjusted using a linear model in which the slope of the curve was modeled as a function of

temperature and RH. Linear regression analysis using R software version 4.4.2 was used to estimate the survival of *S. enterica* in carrots samples in two different intervals: the first interval was the first 24 h of storage and the second interval covered 24 to 168 h. *S. enterica* showed better survival in carrots stored at higher temperature and at higher RH conditions. Temperature and RH significantly influenced survival ( $p < 0.05$ ), but their interaction was not significant ( $p > 0.05$ ). Linear regression analysis showed an excellent fit for the survival rates of *S. enterica* in carrots samples, with an adjusted R<sup>2</sup> value of 0.95 in the first interval and of 0.92 in the second interval. The resulting models for *S. enterica* were  $\text{rate} = -0.2216241 + 0.0136942(T) + 0.0006656(RH)$  for the first interval and  $\text{rate} = -0.04090 + 0.001159(T) + 0.0002441(RH)$  for the second interval. The models developed in this study will be useful for future microbial risk assessments to develop accurate risk analysis for *Salmonella* outbreaks related to minimally processed carrots.

**Keywords:** Minimally processed carrot; *Salmonella enterica*; Mathematical modeling; Linear regression; Temperature; Relative humidity

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## PP04

### Intraspecies Variability in Kinetic Growth Parameters of *Alternaria* Isolates from Oat and Apple

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*Alternaria* spp. is a dominant genus in the mycobiota of cereal grains worldwide, playing a significant role in both crop contamination and food safety concerns. Beyond cereals, *Alternaria alternata* contributes to postharvest spoilage of fruits and vegetables, such as apples, which are prone to mouldy core and soft rot. The common mycotoxins produced by *Alternaria* spp. in food include alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), and tenuazonic acid (TeA). In 2022, the EU Commission issued a recommendation to monitor three *Alternaria* mycotoxins (AOH, AME, TeA) in food, setting maximum limits of 2 µg/kg for AOH and AME, and 500 µg/kg for TeA in cereal-based foods for infants and children. EFSA identifies children as the most at-risk group, highlighting the need for stringent control measures due to their cereal and fruit-rich diet. To address these concerns, this study aimed to assess the intraspecific variability of *A. alternata*, particularly its adaptability to different abiotic conditions and substrates, addressing the limited data on the *Alternaria* ecophysiology. Twenty *A. alternata* strains were isolated from

apples and oats, selected for their toxin production potential. These strains were inoculated on apple and oat agar and incubated at 5, 20, and 35 °C for 28 days, with growth periodically assessed by measuring the colony radius. The Modified Baranyi and Roberts model was used to estimate growth rate and lag time. In addition, intraspecies variability was also assessed in terms of toxin production after 28 days.

Results showed that oat agar supported better growth for both oat-and apple-derived isolates at 5 and 35 °C, whereas apple agar was more favourable at 20 °C. Oat-derived isolates generally exhibited faster growth, regardless of the medium, suggesting no substrate specificity. The highest intraspecific variability was observed at 35 °C on oat medium, where the fastest growth occurred. This suggests that *Alternaria* strains from oats may possess the ability to tolerate high temperature, as would be expected in Southern Europe cereal fields under climate change. Understanding these adaptive traits is key to anticipating mycotoxin risks and optimizing food safety control strategies.

**Keywords:** *Alternaria alternata*; intraspecific variability, mycotoxin production, predictive mycology

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## PP05

### Modelling Thermal Inactivation of *Listeria monocytogenes* in Ground Beef using Mexican Oregano Essential Oil

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The persistence of *Listeria monocytogenes* in meat products poses a significant public health risk<sup>1</sup>. Essential oils (EO), including oregano EO (OEO) are promising natural antimicrobials that could complement thermal processes to control this pathogen. OEO, rich in monoterpenes like carvacrol and thymol, exhibits strong antimicrobial properties. The study aimed to evaluate the combined effect of Mexican OEO and mild heat treatments on *L. monocytogenes* in ground beef. Ground beef (10 g) was treated with two OEO formulations: pure OEO (OP) and fractionated OEO (FIV), both at 0.06% v/v, and subjected to thermal processing at 56 and 60°C. Meat samples, inoculated with *L. monocytogenes* (108 CFU/mL, strain L1.PM1, isolated from clinical listeriosis cases), were collected at intervals (0-15 min for 56°C and 0-5 min for 60°C) and placed in an ice bath. Three repetitions and two independent experiments of each treatment were performed. Microbial counts were used to assess inactivation (Log CFU/mL), and color and sensory analyses were performed over 9-day storage period at 5°C. The Mafart model was fitted to observed data in

Bioinactivation<sup>4</sup> software to mathematically describe the thermal inactivation process. D56°C and D60°C were interpolated to the secondary model from previous in vitro data on a simulated meat medium (SMM). D values from the different treatments were: D56°C =  $0.39 \pm 0.17$ ,  $0.28 \pm 0.11$  and  $0.23 \pm 0.17$  and, D60°C =  $0.20 \pm 0.16$ ,  $0.05 \pm 0.03$  and  $0.03 \pm 0.02$  min for control, OP and FIV, respectively. Results indicated that the addition of OEO enhanced the thermal inactivation of *L. monocytogenes* compared to the control. Using FIV, inactivation levels of  $3.00 \pm 0.11$  Log CFU/mL were reached at 56°C after 2.5 min of treatment while the control group reached  $1.91 \pm 0.30$  Log CFU/mL under the same conditions. Sensory analysis showed no significant color changes, and while acceptability declined over time, OEO treatments maintained higher stability through storage. In conclusion, sublethal concentrations of fractionated Mexican OEO could enhance thermal inactivation of *L. monocytogenes* in ground beef offering a potential strategy for improving food safety and product stability.

## PP06

### Exploring the antimicrobial resistance profile of *Staphylococcus aureus* isolated from Portuguese fermented meat products

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Chouriça and alheira are traditional Portuguese sausages with culinary, cultural, and economic significance. However, their artisanal production is often linked to non-standardised manufacturing processes that may result in contamination by foodborne pathogens and antimicrobial resistance (AMR) dissemination.

In this study, forty-seven *Staphylococcus aureus* isolates originating from Portuguese fermented sausages (chouriça, n=25; alheira, n=22) were subjected to antimicrobial susceptibility testing by disk diffusion following EUCAST guidelines, against 14 antibiotics: benzylpenicillin (1U), cefoxitin (30 µg), norfloxacin (10 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), linezolid (10 µg), rifampicin (5 µg), trimethoprim (5 µg), trimethoprim-sulfamethoxazole (1.25-23.75 µg) and chloramphenicol (30 µg). The resulting data were analysed performing proportion analysis of resistance and Principal Component Analysis (PCA) using the AMR package implemented in the R software.

Antibiotic resistance was detected against benzylpenicillin, norfloxacin, gentamicin, tobramycin, erythromycin, clindamycin, tetracycline and trimethoprim. Thirty-three isolates (70.2%) showed resistance to at least one antibiotic, and one isolate from chouriça was multidrug-resistant (MDR) (2.1%). Cefoxitin susceptibility of all strains indicates the unlikely

probability of methicillin-resistant *S. aureus* occurrence. Benzylpenicillin resistance was the most frequently detected resistance (chouriça, 50%; alheira, 40%), followed by erythromycin (chouriça, 40.9%; alheira, 16%), while norfloxacin resistance was exclusive to alheira isolates (17.4%).

PCA generated two components, explaining 70% of the variance. PC1 (53.0%) was strongly correlated to resistance to gentamicin, trimethoprim, and tetracycline, moderately to tobramycin and less with erythromycin. Still, all these antibiotics were closely associated together, suggesting shared resistance mechanisms, either through MDR mechanisms, efflux pump systems, target site modifications or enzymatic modifications. PC2 (17.0%) was positively associated with norfloxacin and negatively with benzylpenicillin and clindamycin, with no positive association one to another. Furthermore, the projection of scores revealed distinct clustering of alheira and chouriça strains, indicating different resistance profiles.

Detection of antibiotic-resistant *S. aureus* in fermented sausages, particularly to antibiotics commonly used in human treatment, probably results from poor hygiene practices during manufacturing and/or improper fermentation, allowing the survival of human-derived strains in the end product. Future research will focus on resistance transmission and genotypic characterization.

## PP08

### Understanding *Pseudomonas* spp. to Minimise Economic Losses in Artisanal Cheese Production

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*Pseudomonas* spp. causes significant economic losses in the Spanish dairy sector due to spoilage effects that alter flavour, texture, and appearance of cheeses. Its ubiquity and psychrotrophic nature make prevention especially challenging. Microbial modelling is a key tool for developing effective risk mitigation strategies.

This study aimed to evaluate the behaviour of *Pseudomonas* spp. isolated from artisanal cheesemaking environments and assess their response to seven different starter cultures through challenge testing in goat milk and fresh cheeses.

*Pseudomonas* isolates ( $-80^{\circ}\text{C}$ ) were reactivated (BHI broth,  $30^{\circ}\text{C}/48\text{h}$ ) and evaluated for growth through optical density (OD540) measurements. Cultures ( $100\mu\text{L}$ ) were inoculated into 2mL of pasteurized milk and incubated at  $8^{\circ}\text{C}/7$  days in 24-well microplates. Colour changes in milk were also assessed as part of phenotypic profiling. Five selected strains were used to inoculate milk for cheesemaking. Fresh cheeses were stored for 30 days and classified as: CONTROL (no inoculum/culture), PSE (*Pseudomonas* spp. inoculation) and PSE+LAB (*Pseudomonas* spp. and starter culture). Samples were incubated at  $4-8^{\circ}\text{C}$  and sensory and microbiologically analysed within 24-72h.

Molecular identification (16S rRNA) was used to monitor microbial dynamics at different stages of cheese storage.

A total of twenty *Pseudomonas* spp. strains were initially evaluated, isolated from altered cheeses, intermediate products, processing facilities and equipment. The initial concentrations of the different strains in each well ranged from 0.96-2.36 log CFU/mL. Based on their growth profiles, *P. gessardii*, *P. putida*, and *P. fluorescens* were selected for challenge testing, showing concentrations increases ranging from 6.95 to 7.81 log CFU/mL. *P. putida* induced a strong blue discolouration in the milk, while *P. gessardii* caused a yellow colour change. In PSE+LAB samples, an expected reduction of 5 log CFU/g in *Pseudomonas* spp. counts was observed. From these results, three starter cultures were selected for further challenge testing, with growth rates in cheeses ranging 0.009-0.050 log CFU/h.

This study highlights the impact of dominant *Pseudomonas* spp. strains on spoilage in artisanal cheesemaking, while supports the use of selected starter cultures as effective mitigation strategy and the integration of phenotypic, microbial and molecular data for reducing economic losses and improving product quality.

## PP09

### Quinolone-resistance in *Campylobacter* isolates in Europe: systematic review and meta-analyses

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Campylobacteriosis remains the most frequently reported foodborne illness in the European Union, with added public health implications when infections involve antimicrobial-resistant strains by mapping fluoroquinolone-resistant campylobacters from broilers. This systematic review and meta-analysis investigated fluoroquinolone resistance in *Campylobacter* spp. isolated from broiler meat across the Europe at retail or slaughterhouse level. A comprehensive systematic review was conducted following the EFSA guidelines and PRISMA protocol. Meta-analysis was carried out using the 'meta-prop' command in R statistical software. The pooled prevalence of resistant isolates was estimated by analysing variations between subregions (as for United Nation Geographical division), matrices of origin of the isolates (skin, meat or offals), processing stages of sampling (slaughterhouse, processing plant and retail), and *Campylobacter* species. Concerning Europe, out of 54 eligible articles, data on resistance from 9,109 isolates were extracted and analysed. The pooled prevalence of ciprofloxacin (CIP) resistance in *Campylobacter* spp. isolates varied significantly

by subregion, ranging from 0.41 [95% CI, 0.31–0.51] in Western Europe to 0.77 [95% CI, 0.66–0.86] in Southern Europe. Overall, CIP resistance at the European level was 0.63 [95% CI, 0.56–0.69], similar to the one expressed towards nalidixic acid. Moreover, change in the resistance levels were observed over the period of last 20 years and a rising trend was seen for both ciprofloxacin and nalidixic acid. This study highlights the widespread prevalence of fluoroquinolone-resistant *Campylobacter* isolates in broiler meat at retail in Europe, with subregional variations. These results are in line with the recent EFSA and ECDC AMR Summary Reports and emphasize the need for continued AMR monitoring and targeted interventions to mitigate resistance within the food chain. The next step will be the comparison of the European region with other regions worldwide in order to have a global scale overview and highlights data gaps and crucial evidences that can support the lead to effective interventions against AMR and foodborne diseases.

## PP10

### Assessing the decontamination efficacy of photodynamic inactivation against *Alicyclobacillus acidoterrestris* spores on fresh orange surfaces: A kinetic study.

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**Introduction:** The presence of *Alicyclobacillus acidoterrestris* spores may cause fruit juice spoilage main due to guaiacol production since pasteurization is not able to inactivate the spores.

**Purpose:** The investigation explores the efficacy of antimicrobial photodynamic treatment (APDT) using new methylene blue N (NMBN), methylene blue (MB), curcumin (CURC), 8-methoxypsoralen (8-MOP), and chlorin e6 (Ce6) as photosensitizers (PS) in the presence and absence of potassium iodide (KI) as a potentiator agent against *A. acidoterrestris* spores.

**Methods:** A spore suspension of *A. acidoterrestris* (DSM 2498) was prepared in Roux bottles containing YSG agar (pH adjusted to 3.7) supplemented with 10 ppm manganese sulfate. Stock solutions of the photosensitizers were prepared using sterile deionized water (for NMBN and MB) or DMSO (for CURC, 8-MOP, and Ce6). The *A. acidoterrestris* spore suspension, adjusted to  $10^3$  spores/mL, was exposed to simulated full-spectrum solar radiation using a Xenon Test Chamber Q-SUN (Model XE-3-HC). A minimal inhibitory concentration (MIC) assay, conducted in triplicate, was performed to determine the optimal conditions (light exposure

time and photosensitizer concentration) for APDT prior to application on oranges artificially inoculated with *A. acidoterrestris* spores. Light and dark controls were included to evaluate the isolated effects of radiation and photosensitizers, respectively. Survival curves were generated by plotting logarithmic population counts ( $\log_{10}$  spores/mL) against fluence ( $J/m^2$ ). Inactivation data were analyzed using the GlnaFit Excel® add-in.

**Results:** The MIC assay results demonstrated that combining NMBN, MB and 8-MOP with full-spectrum solar radiation achieved the inhibition of *A. acidoterrestris* spore germination only in the presence of KI. Conversely, when CURC and Ce6 was tested under identical conditions, the full-spectrum solar radiation exhibited inhibitory potential against *A. acidoterrestris* spores both in the presence and absence of KI. Subsequent experiments on orange surfaces will apply concentrations of 50–100  $\mu$ M of NMBN, 100–150  $\mu$ M of MB, and 10–25  $\mu$ M of 8-MOP (with KI), 10–25  $\mu$ M of CURC, and 10–50  $\mu$ M of Ce6 (with and without KI).

**Significance:** Results obtained in this study will contribute to future APDT developments for field applications.

## PP11

### Understanding and Modeling Salmonella Single-Cell Behavior to Acid Stress in Food Preservation

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Food safety is a fundamental issue for consumers and food industry and, lately, the need for a new consideration for the preservation techniques targeting on high quality and mild processing is highlighted. Thus, low pH stress becomes one of the most valuable tools for pathogens control. The knowledge of only the average population decline is unlikely to be a sufficient basis for processing design and, most importantly, for pathogens such as Salmonella with low infectious doses. The aim of this study was to investigate the inactivation behavior of Salmonella enterica ser. Agona under acidic food preservation stress focusing on addressing low pH single cell inactivation as well as its implication on population dynamics but also the mechanisms underlining single cell behavior. A direct microscopic time lapse method was developed, using appropriate staining for cell viability. Salmonella cells were exposed to various acidic conditions and the actual inactivation times of the cells in a population

were estimated. Furthermore, fluorescently tagged strains employed for monitoring gene expression with relevance to acidic conditions and combining it with the inactivation behavior at the phenotypic level. The time of inactivation of single cells was highly heterogeneous as well as the gene expression pattern of individual cells. Individual cell inactivation times were fitted to a variety of continuous distributions. The best fitted distribution was further used to predict the inactivation of Salmonella populations of various initial levels using Monte Carlo simulation. The simulation results showed that the variability in inactivation kinetics is negligible for concentrations down to 100 cells. The direct assessment of individual cell inactivation behavior has the potential to increase the accuracy in risk assessment models as well as to be the basis of stochastic microbial inactivation models for the development and improvement of risk-based designs and food safety management systems.

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## PP12

### Development of a probabilistic mycotoxin (DON) exposure assessment in pita bread: a case study of Greece

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Flatbreads constitute a major food consumed worldwide. However, deoxynivalenol (DON) remains one of the most frequently mycotoxins produced by *Fusarium* species, due to the wide occurrence in highly consumed cereal-based food and its associated toxicological effects. The aim of this study was to assess the exposure of Greek population to DON related to the consumption of pita bread as a case of flatbread product through a probabilistic approach.

Data of 710 individuals across all age groups consuming pita bread were retrieved from the Hellenic National Nutrition and Health Survey (HNNHS) covering all geographical regions of Greece. The mean occurrence value (mean contamination level of mycotoxin), based on median DON concentrations, was 43.5 µg/day for unleavened bread, crisp bread and rusk according to EFSA. Consumption and occurrence data were both codified according to the FoodEx2 classification system developed by EFSA. Dietary chronic exposure to DON was assessed at individual level by multiplying the

average daily consumption of pita bread with the mean DON occurrence dividing the results by the individual's body weight. The mean and 95th percentile dietary exposure to DON were estimated from the distribution of the individual exposure results. Dietary exposure assessment was performed using ImproRisk model V0.5.4.

The distribution of exposure to DON, based on median occurrence scenario, showed that the tolerable daily intake (TDI=1 µg/kg bw/day) was not exceeded for the Greek population. It was also found that the population groups of toddlers, other children, and adolescents had the highest exposure values after consumption of pita bread. However, Hazard Quotient for these population groups was still less than 1, indicating the health risk of the intake level is acceptable.

The current results suggest pita bread marketed and produced in Greece presents low risk for average and excessive consumers regarding the content of mycotoxin DON.

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## PP14

### Estimating the Relative Risk Associated with Stress-Resistant Variants of Salmonella: UV-treated Orange Juice as a Case Study

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The ability of Salmonella cells to resist and adapt to adverse conditions encountered in food and food processing environments –such as acid– is one of the main characteristics that have made this microorganism such a relevant health hazard. The impact of these responses alone has already been reviewed elsewhere, but with the emergence of variants with increased cross-resistance to food processing technologies and agents, it is also important to investigate the implications of an altered genotype-phenotype in a complex scenario that involves not only the application of such technology in the food chain, but all the successive steps with the potential to cause an altered response and, consequently, an altered final risk associated with Salmonella in food.

Therefore, the aim of this work was to estimate the relative risk of two Salmonella enterica serovar Typhimurium isolated variants with increased resistance to acid (SL-Acid) and UV-C light (SL-UV) –as compared to their parental strain– using UV-treated Orange Juice as a Case Study. For this, the fitness of these Salmonella strains was evaluated throughout a simplified modular process consisting in: 1) pre-treatment

storage, 2) UV-C treatment, 3) post-treatment storage, 4) ingestion-digestion of the orange juice, and 5) final invasion of Caco-2 cells.

Based on the results obtained, two deterministic (with or without interactions between modules) and a probabilistic (MonteCarlo) model were constructed in order to evaluate the relative risk associated with each strain in several pre-established scenarios. Experimental results revealed the existence of interactions between the modules. It was also found that the introduction of these interactions in the models was necessary for an adequate prediction of the behavior of the different Salmonella strains. Likewise, it was possible to design a probabilistic model capable of making estimations of the relative risk associated to each strain in more realistic scenarios. Altogether, results obtained indicate that the resistant variants would pose a higher risk than the parental strain in orange juice, and that the SL-UV variant (the most resistant to the processing technology but also to citric acid) was not the one presenting the greatest final relative risk in any of the cases studied.

## PP15

### Modeling and Optimization of Polyphenol Extraction from *Fucus vesiculosus*: A Comparative Study of HAE, UAE, and PLE

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In recent years, macroalgae has been gaining relevance as a new source to obtain active compounds with potential use in biotechnological processes and develop new food products. Their metabolic diversity includes molecules with biological properties, such as polysaccharides, proteins, fatty acids, vitamins and minerals. Polyphenols, also present, are usually less studied because of their complex structure and identification. This study aimed to model and optimize the extraction of polyphenols from *Fucus vesiculosus*, with a special focus on phlorotannins by different extraction techniques: heat assisted extraction (HAE), ultrasound assisted extraction (UAE) and pressurized liquid extraction (PLE). For this purpose, response surface methodology (RSM) was applied using a circumscribed central composite design (CCCD) with five levels and three variables (X1, time; X2, temperature or power and X3, ethanol percentage) and fitted using the least squares method with a polynomial equation based in the Box-Behnken design. The optimization was performed

considering extraction yield and phenolic compounds quantified by HPLC-ESI-QqQ-MS/MS as total tannins, total bromophenols and total phenolic acids, used as global indicators, together with 9 compounds that corresponded to the three most abundant in each class. Totally, 79 polyphenols were identified and tannins stood out as the most frequent compounds with 45 representatives. PLE was shown to be the most effective technique in terms of improved extraction of active compounds, ranging from 9.1 to 63.4%. The highest extraction efficiency for tannins, bromophenols, and phenolic acids was observed under extreme conditions. The techniques studied were effective in maximizing the yield of polyphenols from *F. vesiculosus* and PLE proved to be superior. The use of RSM to optimize extraction conditions further enhances the efficiency and sustainability of the extraction process. Overall, the study results support the continued exploration and utilization of marine resources to address global challenges related to health, nutrition, and environmental sustainability.

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## PP16

### Spectroscopy-based tools and their predictive capacity of raw ovine milk quality and hygiene

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Milk and its products are among the most valuable commodities. Consumer-driven demand for healthy, safe, and premium-quality dairy products constantly motivate the dairy industry to update its standards and reshape the structure of the milk supply chain and its processing procedures. This ensures the desirable intrinsic and extrinsic quality traits of the products, with raw milk quality and hygiene being recognized as fundamental components of this effort. The aim of this study was to assess the use of spectroscopy-based technologies, as rapid analytical tools for estimating fat, protein, lactose, and total solids contents, as well as, total bacteria count (TBC) and somatic cell count (SCC) in ovine milk, at the point-of-service. Except for the milk hygiene traits, critical udder health indicators were also assessed at the animal level. Udder health data and milk samples from 300 individual adult dairy ewes were collected from a commercial farm and analyzed using Ultraviolet/Visible (UV/Vis), Near-Infrared (NIR), Raman and Attenuated Total Reflectance (ATR-FTIR) spectroscopy tools.

Fourier Transform Infrared (FTIR) was used as the reference method for the analysis of fat, protein, lactose, and total solids contents, while flow cytometry was the reference method for TBC and SCC. The collected spectra were further analyzed and comparatively assessed regarding their capacity to predict udder health status and milk quality and hygiene traits, while evaluating interrelationships between these traits and the collected spectral data. For this reason, various statistical models were developed and assessed. Herein, we present the analytical protocols and procedures, the most efficient algorithms, and some promising preliminary results of the predictive capacity of the studied spectroscopy tools as derived by the spectral analyses and the subsequent development and assessment of the statistical models. Additionally, the factors challenging the predictive performance of the tools regarding milk quality traits and microbial and cellular parameters in the ovine milk are discussed based on evidence.

## PP17

### Modeling the temperature effect on the growth of uropathogenic *Escherichia coli* (UPEC) on roasted duck meat

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Uropathogenic *Escherichia coli* (UPEC) in ready-to-eat (RTE) meat products has been confirmed, suggesting that meat may serve as a potential reservoir. UPEC is also detected in roasted duck, a popular traditional Taiwanese dish. Since RTE products are consumed without reheating, contamination with UPEC poses a food safety risk. Thus, this study aims to develop a growth model for UPEC in roasted ducks, simulating its growth dynamics at different temperatures and proposing preventive measures.

This study categorized UPEC strains into clinical isolates (reference strain) and food isolates (obtained from roasted duck and identified in this study). A cocktail of clinical or food isolates, each carrying different virulence genes, was inoculated onto sterile roasted duck breast meat and incubated at 4°C to 40°C. The growth kinetics of UPEC were analyzed using primary and secondary models in the IPMP software to evaluate temperature effects on maximum specific growth rate and lag phase duration.

Results indicated that UPEC exhibited optimal growth at 20°C, 35°C, and 40°C, with food isolates displaying a higher specific growth rate ( $\mu_{max}$ ) and reaching significantly higher

maximum bacterial counts ( $y_{max}$ ) than clinical isolates at 35°C (food:  $\mu_{max} = 1.89$ ,  $y_{max} = 21.79$  ln CFU/g; clinical:  $\mu_{max} = 1.8$ ,  $y_{max} = 19.03$  ln CFU/g) and 40°C (food:  $\mu_{max} = 3.49$ ,  $y_{max} = 20.41$  ln CFU/g; clinical:  $\mu_{max} = 1.97$ ,  $y_{max} = 18.48$  ln CFU/g). These findings suggest food isolates possess greater adaptability and growth potential in roasted ducks, particularly under elevated temperatures. This underscores the potential risk of UPEC proliferation in improperly stored roasted duck and highlights the critical need for stringent temperature controls to mitigate food safety risks.

To minimize UPEC contamination risk, temperature control is essential during roasted duck preparation and storage. However, our findings indicate that at relatively higher temperatures (35°C and 40°C), food isolates exhibited significantly faster growth and higher  $y_{max}$  than clinical isolates. Therefore, predictive models should not rely solely on clinical isolate data, as this may underestimate UPEC growth in food, leading to a fail-dangerous scenario. Incorporating data from food isolates is crucial for improving accuracy of predictive models and enhancing food safety risk assessments.

## PP18

### Assessment of the Variability in the Microbiological Quality of Four Batches of Raw Milk Cheese Produced from Milk Supplied by Two Different Farms in Italy

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In this study, we investigated the correlation between the microbiological parameters of raw milk produced by two different farms in Northern Italy and those of the corresponding raw milk cheeses. Four batches of cheese were analyzed throughout the production process, up to the end of the seasoning period. The microbiological parameters examined included Total Mesophilic Bacteria (TBC), Enterobacteria (ENT), and Lactic Acid Bacteria (LAB). Additionally, pH and water activity (aw) were measured.

The distribution and homoscedasticity of these parameters were assessed across the four tested batches at different time points (i.e., 7, 15, and 30 days of seasoning). Statistical differences between the microbiological and physicochemical parameters of the cheese batches and raw milk were evaluated using ANOVA, robust ANOVA, and the Kruskal-Wallis test based on the above-mentioned assumptions.

Furthermore, the evolution of the microbial population in each batch was analyzed using paired sample time-series analysis, employing ANOVA for repeated measures, the Conover test, and the Durbin test for unpaired block design. Notably, we identified statistically significant differences ( $p \leq 0.05$ ) in TBC, ENT, LAB, pH, and aw among the four batches throughout the entire production process, from raw milk to cheese aged for 30 days. The only exception was the pH of cheese seasoned for 15 days, which did not reach statistical significance ( $p = 0.0659$ ).

Additionally, post-hoc tests, including Tukey's HSD, Tamhane's T2 test, Dunn's test, pairwise t-tests, the Conover all-pairs test, and the Durbin all-pairs test, were applied to determine where significant variability existed among the four analyzed batches.

Finally, correlations among the variables collected at each follow-up were analyzed using the Spearman approach to provide a comprehensive time-series overview.

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## PP19

### Nitrite Reduction in Cooked Pork Ham: A Risk for Food Safety?

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Cooked pork ham is formulated with nitrite, an authorised preservative that, combined with other hurdles, has been used for decades to ensure the microbiological safety of cured meat products. However, the presence of nitrites in foods can lead to the formation of nitrosamines, some of which are carcinogenic [1]. For this reason, revised regulation of the European Commission defines maximum levels of 80 mg of nitrite per kg of meat [1].

Given this, the aim of this research was to evaluate how reducing nitrite levels in cooked ham, from 150 ppm (standard product) to 80 ppm, affects the behaviour of *Listeria monocytogenes* (LM). For that, slices (10-12 g) of the two formulations of ham were inoculated with a cocktail of seven LM strains (~4.5 log CFU/g), packed under MAP (80% N<sub>2</sub>, 20% CO<sub>2</sub>) and stored at 4 °C and 8 °C for 35 days. At specific days, LM was enumerated in PALCAM agar, and the Baranyi and Roberts model was

used to describe pathogen growth. Two independent replicates were carried out.

At either of the temperatures tested, the reduction of nitrite from 150 ppm to 80 ppm did not reveal a considerable impact on the maximum growth rate of the pathogen ( $\mu_{max}$ ; log CFU/g day<sup>-1</sup>). At 4 °C,  $\mu_{max}$  was  $0.071 \pm 0.011$  (150 ppm) and  $0.068 \pm 0.016$  (80 ppm). At 8 °C, the maximum rate was  $0.111 \pm 0.019$  (150 ppm) and  $0.117 \pm 0.017$  (80 ppm). These results suggest that an increase of 4 °C in temperature has a bigger impact on LM growth rate in cooked ham than a reduction of ~46% in its nitrite content.

Reformulation of any food product requires careful consideration to ensure that food safety is maintained. In this case, it appears that reducing nitrite levels by nearly 50% does not promote the growth of LM in cured pork meat. This is an encouraging finding for the cured meat industry, as it supports the potential for reformulating products with lower nitrite levels.

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## PP20

### L. Monocytogenes Growth Simulation Based on Temperature Distribution Mapping Within A Beef Dry-Aging Chamber

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Beef dry aging is a process aimed at enhancing meat tenderness and flavor. Commission Delegated Regulation (EU) 2024/1141 establishes specific conditions for dry aging, including a surface temperature between 0.5 and 3°C, a maximum relative humidity of 85%, an airspeed of 0.2–0.5 m/s, and a maximum duration of 35 days. Food business operators must demonstrate compliance with these conditions or validate microbiological safety when using alternative parameters, including extended aging times.

This study assessed key parameters affecting dry-aged beef safety. Experimental data was collected in the chamber (temperature and relative humidity (RH)), and on the surface of beef (pH and water activity (aw)) aged up to 55 days in a chamber set to 1.5 °C and 65 %RH. The recorded temperature and humidity data were used to perform a chamber mapping, that revealed considerable spatial and temporal variability. In fact, despite the settings, actual conditions fluctuated significantly, highlighting the challenge of maintaining uniform environmental conditions. Temperature ranged from 1.8°C to 4.2°C, while RH varied between 53.2% and 99.9%. Based on this analysis, three scenarios were defined: (1) worst-case (highest

temperature profile); (2) best-case (lowest temperature profile); and (3) controlled (constant 1.5°C). The impact of these conditions and of meat physicochemical characteristics on *Listeria monocytogenes* growth was evaluated using the predictive model by Mejlholm & Dalgaard (2009), calibrated for beef aging (EFSA BIOHAZ et al., 2023). Results indicate that *L. monocytogenes* can proliferate significantly during the dry-aging process, reaching its maximum (~9 Log CFU/g) in the worst-case scenario. Even under temperature-control (1.5°C), microbial growth remains a concern, likely due to the relatively high surface aw observed in this study (0.981). A faster aw decline <0.950 could have significantly restricted or prevented pathogen growth (Panella-Riera et al., 2021). Therefore, temperature control alone is insufficient to ensure safety, and a multi-factorial approach is necessary, precisely controlling RH and airflow. Although legislation sets a maximum RH of 85%, our results highlight the need for dynamic humidity control to enhance surface drying. Optimizing RH and airflow to accelerate meat surface aw reduction is crucial for limiting pathogen growth and ensuring a safe dry-aging process.

## PP21

### Molecular Identification and Genetic Diversity of Mycotoxigenic Fungi in Commercial Maize-Based Products from the Greek Market

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Maize (*Zea mays* L.) plays a pivotal role in the European Union's agricultural sector, ranking as the second most cultivated cereal. With an average annual production exceeding 65 million tons over the past five years, it significantly contributes to regional food security and economic stability. However, maize and maize-based products are highly susceptible to fungal contamination, particularly by species capable of producing toxic secondary metabolites, including mycotoxins. These contaminants pose a significant concern in the food industry due to their potential detrimental impact on human health. In this study, 160 commercially available maize-based samples from the Greek market were analyzed to identify the predominant fungi responsible for contamination. Microbiological techniques were employed by culturing samples on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Rose Bengal Chloramphenicol Agar (RBC). Fungal colonies were visually examined for morphological characterization based on colony features, providing a preliminary identification of the species. A total of 62 isolates associated with mycotoxin-contaminated products were further analyzed using molecular techniques for taxonomic identification. Molecular identification was conducted through sequencing of the internal transcribed spacer

(ITS) region, followed by nucleotide-nucleotide BLAST analysis for taxonomic confirmation (species identification considered reliable at >97% sequence similarity). ITS sequencing revealed the occurrence of eleven fungal genera: *Aspergillus* (13 isolates), *Cladosporium* (4), *Fusarium* (9), *Hamigera* (1), *Mucor* (6), *Paecilomyces* (4), *Penicillium* (9), *Phanerochaete* (4), *Rhizopus* (7), *Sarocladium* (1) and *Talaromyces* (4). Particular attention was given to *Aspergillus*, *Fusarium*, and *Penicillium* due to their well-documented mycotoxin production. In these cases, sequencing of the calmodulin genes was performed to enhance genetic diversity assessment and improve identification specificity. Notably, several identified species, including *Aspergillus fumigatus*, *Aspergillus clavatus*, *Aspergillus ustus*, *Fusarium verticillioides*, and *Talaromyces variotii*, have been previously reported as potential mycotoxin producers. These findings highlight the need for continuous monitoring, rigorous quality control, and improved fungal detection methods in maize-based products to mitigate potential health risks. Further research is essential to assess the ecological diversity of fungal communities and their implications for food safety.

**Acknowledgement:** This project has received funding from the European Union's Horizon Europe Research and Innovation Programme under Grant Agreement No 101079173.

## PP22

### Growth of two *Listeria monocytogenes* strains, persistent and non-persistent: effect of temperature

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Two strains persistent and non-persistent were selected and grown in different media to gain reliable quantitative growth characteristics. In this study, the effect of temperature in the range from 6 °C to 43 °C on the planktonic growth of genotypically and phenotypically different strains LM9611-19 (LM-P, persistent) and LM120/5 (LM-S, sporadic - potentially non-persistent) in Tryptone Soy Broth (TSB) and in semi-synthetic cheese medium (SCM) was investigated. Two steps of growth modelling were applied to primary growth data and growth parameters using Baranyi and cardinal temperature models (CM), respectively. No statistically significant differences were found between the growth rates of the strains within the temperature range of 6 °C – 37 °C in both media. However, the average growth rates were significantly higher ( $p < 0.05$ ) for LM-P than for LM-S at 40 °C and 43 °C in both media. Regardless of whether calculated on  $\mu_{max}$  or  $\lambda$  basis, in TSB or SCM,  $T_{min}$  for LM-P strain ranged

from -1.2 to 0.7 °C with an average of  $0.0 \pm 0.9$  °C (mean  $\pm$  SD). Other averages of cardinal values were in TSB ( $a_w = 0.995$ ; pH 7)  $T_{opt} = 37.8 \pm 2.0$  °C,  $T_{max} = 43.6 \pm 0.5$  °C and  $\mu_{opt} = 1.27 \pm 0.2$  h<sup>-1</sup>. In SCM ( $a_w = 0.970$ , pH 7), the averages of  $T_{opt}$ ,  $T_{max}$ , and  $\mu_{opt}$  were  $38.0 \pm 1.2$  °C,  $45.2 \pm 2.9$  °C and  $0.92 \pm 0.04$  h<sup>-1</sup>, respectively. Generally, the parameters of the CM model for the growth rate of sporadic strain in cheese medium were lower than for the persistent strain. This also includes  $\mu_{opt}$ , which reflects lower experimental growth rates from  $T_{opt}$  to  $T_{max}$ . However, based on the results found in the suboptimal temperature range, the growth rate did not play an important role in the persistency characteristics. To consider the ComBase Predictor data ( $n = 8$ ), the bias factors (Bf) of 1.08 and 1.05 and accuracy factor (Af) of 1.09 and 1.07 were calculated for the LM-P strain and LM-S strain, respectively. The study revealed different growth responses in temperatures higher than  $T_{opt}$ .

#### Acknowledgements

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## PP23

### Growth of *Bacillus cereus* and cereulide production in UHT plant-based milk alternatives

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Limited knowledge is available on the ability of growth and toxin formation of *B. cereus* in plant-based milk alternatives. This information is necessary to confirm whether the current production practices designed to control the safety of dairy products can be applied to plant-based products. This study presents a comparison of the growth of *Bacillus cereus* and cereulide production in various matrices, including bovine milk, oat-based milk, pea-based milk, and a hybrid of fava bean and bovine milk concentrate.

Each matrix was inoculated with emetic *B. cereus* F4810/72 strain at an initial level of 2 log CFU/ml and stored at constant temperatures ranging from 10 °C to 50 °C. Samples were taken at specified time points for bacterial enumeration via plate counts and cereulide analysis using LC-MS/MS. The cardinal model was used to describe how the growth rates and

the time for first cereulide quantification vary with temperature. Independent data were used for growth and cereulide models' validation at 35 °C.

*B. cereus* growth and cereulide production was observed in all studied matrices within the temperature range of 12 °C to 42 °C. Notably, the oat-based milk exhibited the highest growth rate for *B. cereus*, while the hybrid concentrate showed the least growth. Cereulide production was observed to occur later in the hybrid matrix compared to other matrices at temperatures from 12 °C. The established and validated models enable independent predictions of *B. cereus* growth and cereulide production across a broad temperature range, which can be used to evaluate current industrial practices. These findings underscore the potential for implementing targeted control measures in HACCP plans for plant-based products.

## PP24

### Modeling of Mycotoxin Degradation in Foods by application of Cold Atmospheric Plasma: Case studies in wine and apple juice

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Cold atmospheric plasma (CAP) has emerged as a promising technology for the degradation of mycotoxins such as ochratoxin A (OTA) and patulin (PAT). OTA is a toxin frequently detected in wine, while PAT is commonly found in apple juice, both causing health risks. The application of CAP for detoxification can follow two primary approaches: indirect immersion into plasma activated water (PAW) and direct plasma exposure. The indirect method involves the treatment of fruits like grapes and apples in PAW before processing into wine or juice. In the direct method, the final products-wine and juice-identified with excessive toxin levels are treated with plasma.

This study evaluated CAP's effectiveness in degrading OTA in wine and PAT in apple juice while assessing product quality. A kinetic modeling approach optimized CAP treatment conditions. Synthetic wine (tartaric acid 3.5% w/v, ethanol 12% v/v, pH 3.5) was spiked with 5 ppb OTA. Similarly, synthetic apple juice spiked with 100 ppb PAT (13°BRIX, pH 4) was also formulated. Plasma treatments were assessed using a pin-to-liquid DBD reactor, with various voltages (21-27kV for OTA, 19-25kV for PAT) and

duration (1-4 min for OTA, 20s-6 min for PAT). All wine and juice samples were evaluated for OTA and PAT quantification respectively. H<sub>2</sub>O<sub>2</sub> and NO<sub>2</sub>-/NO<sub>3</sub>- concentrations, pH, conductivity as well as quality characteristics (total phenolic content, antioxidant activity, color and acidity) were also evaluated.

A second-order multiparametric model was developed to calculate PAT and OTA concentration in standard solutions for different concentrations of H<sub>2</sub>O<sub>2</sub> and NO<sub>2</sub>-/NO<sub>3</sub>- concentrations produced by CAP. Results showed that OTA degradation reached nearly 65% for treatments exceeding 26kV and 3 min. PAT concentration was reduced by approximately 80% after 1 min of CAP exposure at 21kV. CAP treatment increased significantly RONS concentration in both wine and juice, promoting detoxification and preserving quality attributes including color, phenolic content, antioxidant activity and acidity.

CAP is a viable novel approach for mycotoxin degradation in wine and apple juice without compromising quality while simultaneously ensuring safer consumption.

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## PP25

### Simulation of cross-contamination and re-contamination of uropathogenic *Escherichia coli* (UPEC) in roasted duck meat during retailing

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Roasted duck is a popular traditional food in Taiwan and many Asian countries. Although the preparation process involves high-temperature roasting, microbial contamination remains a concern due to post-roasting handling practices. Notably, two severe foodborne outbreaks related to roasted duck in Taiwan, which led to widespread illness and hospitalizations, were attributed to *Salmonella* cross-contamination during handling. Recognizing the potential microbial risks associated with the preparation of roasted duck, we sampled commercial roasted duck in northern Taiwan. Results revealed the presence of an unexpected bacterial pathogen—Uropathogenic *Escherichia coli* (UPEC), a primary cause of urinary tract infections (UTIs) affecting around 150 million people globally each year. Given the presence of UPEC and the post-roasting handling, it is essential to investigate UPEC cross-contamination and its potential exposure risk to consumers. To simulate the real situation of microbial contamination, four UPEC strains were used in this study to form a bacterial cocktail, including two clinical strains (BCRC 10675 and 15480) and two food isolates obtained and

identified in this study. Duck meat (25 g) was inoculated with 250  $\mu$ L of the bacterial cocktail and left for 30 minutes. Then, cross-contamination scenarios were simulated using cutting boards made of different materials (wooden and plastic) and various types of gloves (white cloth, polyethylene (PE), nitrile butadiene rubber (NBR)) to compare their effect on UPEC transfer. Results showed higher UPEC transfer on wooden boards (73.9%) than on plastic (66.9%). The transfer rates from duck meat to wooden cutting boards ranged from 67–76.6%, while glove transfer rates were 71.6% (white cloth), 65.6% (PE), and 68.8% (NBR). Additional experiments showed that UPEC transfer from contaminated cutting boards to clean duck meat reached 100–109% of the initial bacterial load, suggesting that bacteria can persist and potentially accumulate on wooden surfaces. The overall transfer rate ranged from 65 to 76.6%, indicating a high cross-contamination potential of UPEC. These findings can inform the selection of appropriate food contact materials and the implementation of effective sanitation measures to mitigate microbial risks for both handlers and consumers.

## PP26

### Characterization of a *Listeria Monocytogenes* Reference Strain in View of its Use for Shelf-Life Predictions: Focus on Temperature-Dependent Growth and Cardinal Values

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The European Union Reference Laboratory (EURL) for *Listeria monocytogenes* (Lm) has developed a collection of 30 Lm strains, each characterised by their maximum specific growth rate ( $\mu_{max}$ ) under various broth conditions. These strains were selected based on their origins (meat, dairy products, seafood, environment, and vegetables) and their ability to grow rapidly at cold temperatures (8°C), low pH (pH = 5), and low water activity ( $a_w$  = 0.95). As such, these strains are recommended for use in challenge testing.

Commission Regulation (EU) 2024/2895, amending Regulation (EC) No. 2073/2005 regarding Lm, predicts that more companies will need to determine whether their products support the growth of Lm. This can be done through challenge testing or predictive microbiology.

This study further characterises a strain already recommended for challenge testing as a candidate for predictive microbiology research, specifically focusing on temperature. To achieve this, methods for determining the maximum specific growth rate (via plate count and binary

dilution OD-based techniques) were compared, and the impact of biological versus technical replication was evaluated. The maximum specific growth rate was measured at ten different temperatures ranging from 3°C to 40°C. The experiment was performed in triplicate to assess technical variability and repeated at least twice on different experimental days to evaluate biological variability. Technical variability was consistently lower than biological variability across all temperatures. Cardinal values ( $\mu_{opt}$ ,  $T_{min}$ ,  $T_{opt}$ ) were then determined by fitting a secondary growth model to the maximum specific growth data using BioGrowth4 (Garre et al., 2023).

The cardinal temperature values were successfully characterised, and the strain was confirmed as reliable for use in predictive microbiology studies.

Future steps include characterising other EURL Lm strains and assessing additional factors such as pH,  $a_w$ , and inhibitors, to provide a comprehensive set of strains for predictive microbiology research.

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## PP27

### Effect of temperature on growth of four *Aspergillus carbonarius* isolates on a simulated maize-based medium

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*Aspergillus carbonarius* is an ochratoxinogenic species capable of producing ochratoxin A, a highly toxic metabolite with adverse effects on human health. While commonly associated with grapes and vineyards, it has also been reported in maize. Temperature is a key factor influencing fungal growth and mycotoxin production. This study investigated the growth dynamics of four *A. carbonarius* strains, previously isolated from Greek vineyards, under five isothermal conditions (15°C, 20°C, 25°C, 30°C, and 35°C) in vitro. A maize-based nutrient medium was used to assess strain adaptability to a nutritionally distinct substrate, with water activity 0.93 and pH=6.8. Petri dishes (90mm) filled with medium were centrally inoculated with 10 µL of a 107 spores/mL suspension. The experiment consisted of two batches, each with two replicates (n=4). Fungal growth was monitored for up to 75 days through daily diametric measurements. The maximum specific growth rate ( $\mu_{max}$ ) and the lag phase ( $\lambda$ ) were determined using Baranyi model. The highest growth rate was observed at 30°C across all strains. However, estimating the lag phase was challenging due to rapid initial growth, often

spanning only a few hours, which was not captured at 24-hour measurement intervals. ANOVA was performed to assess temperature effects. Results indicated that lower temperatures (15°C, 20°C) significantly reduced growth rate of all strains, with the most pronounced inhibition at 15°C. The highest growth rate was recorded at 30°C, while 25°C produced comparable results with slight reduction. At optimal temperatures, all strains reached the full colony diameter (90mm) within 20 days, whereas at restrictive temperatures, growth required up to 75 days. Additionally, one strain exhibited reduced growth at 35°C, whereas the other three showed no inhibition. The Baranyi model provided an excellent fit as evidenced by high regression coefficients ( $R^2 > 0.99$ ), and low standard errors of fit. The ANOVA results confirmed that temperature and strain identity significantly affected growth ( $P < 0.001$ ). These findings enhance understanding of *A. carbonarius* thermal growth behavior in maize-based substrates, informing risk assessment and mitigation strategies in food and feed production.

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## PP28

### Modelling of the Fermentation of a Substrate Based on Agro-Industrial Residues for Bioethanol Production

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The large-scale production and processing of agricultural commodities worldwide, results in the extensive generation of agro-industrial residues. One of the most abundantly generated residues is lignocellulosic biomass, which despite its ample content in carbon sources and bioactive compounds, is largely unexploited. The objective of the present work was the development and quantitative description of a bioprocess aiming at the valorization of agro-industrial residues as fermentation feedstock for bioethanol production. The fermentation substrate comprised of a mixture of extracts/hydrolysates, derived after appropriate “green” pretreatment (not involving organic solvents or extreme conditions), from pomegranate residues (PRs) and seeded raisins (SRs) at concentrations 60 and 40% v/v, respectively. Both PRs (i.e. pomegranate peels and seeds remaining after juice extraction) and SRs are important agro-industrial residues in Greece, and their joined utilization resulted in a substrate with a reducing sugars’ concentration of ca. 120 g/L glucose equivalent. The substrate was used in three fermentation trials using *Saccharomyces cerevisiae*, and the bioprocess was quantitatively described via model fitting to

the collected experimental data. The developed bioprocess resulted in satisfactorily high bioethanol production, with the maximum attained concentration being  $50.0 \pm 0.6$  g/L. Among the models tested, the Aiba model was evaluated as the most appropriate for the description of the bioprocess. The mean value of the maximum specific growth rate ( $\mu_{max}$ ) of *S. cerevisiae* was estimated to be 0.135 1/h, and the mean value of the bioethanol yield ( $Y_{ps}$ ) was 0.423 g of produced ethanol/g of consumed reducing sugars. Based on the findings of this study, PRs can be efficiently used in tandem with SRs in the development of a sustainable and cost-effective substrate for bioethanol production.

#### Acknowledgements

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#### Acknowledgements

This research has been financed by the Green Fund of the Hellenic Ministry of Environment and Energy, under the funding program “National Environment and Innovation Activities 2022”, Priority Axis “Research & Application”, Project “Sustainable technology for converting pomegranate residues into bioproducts and bioactive compounds” with the acronym “POMEGRANATE”.

## PP29

### A cardinal-type model with interaction to predict the growth and growth boundaries of *Salmonella* spp.

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**Introduction:** *Salmonella* is a leading cause of food-borne illness, and many types of food have been identified as sources of human cases of salmonellosis (including meat, produce and dairy products). To this date, there is no probabilistic model available that account as temperature, pH, aw, lactic acid and for strain variability on the growth and the growth limits of *Salmonella* spp. The objective of this study is to develop such model and test it against well-characterised data in culture medium.

**Material and Methods:** The overall approach is based on a cardinal-type model with an interaction term. Strain variability is accounted for with statistic distributions. Normal or Pert distributed for the strain-dependent parameters (e.g., cardinal temperatures, MICs of lactic acid). These distributions were based on unpublished data and data collected from the literature for individual *Salmonella* strains. The bacterial kinetics and growth boundaries were predicted by means of 1D Monte Carlo simulations. The model predictive ability was assessed on growth/no growth data from Combase and literature (1915 records, including 621 in presence of lactic acid obtained for 7 different serovars). The model

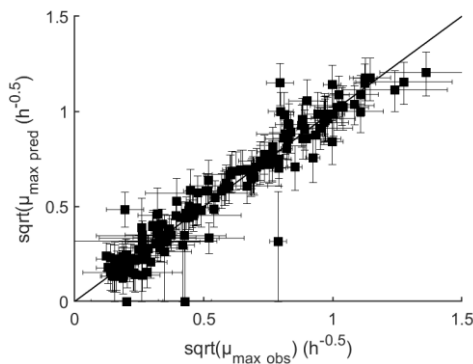
predictions were also compared with 162 maximum specific growth rates from the core Combase FSA-IFR and UTAS database.

**Results:** The predictions of the interaction term for the growth/no growth limits were consistent with experimental observations. The Negative Predictive Value (NPV, number of true negatives divided by the number of predicted “No Growth” conditions) is 99.3%. There is also a good correspondence between the predicted and observed maximum growth rates (92% of overlapping between the 95% confidence intervals on the estimated  $\mu_{max}$ -values and the 95% limits of predictions).

**Conclusion and significance:** This stochastic approach for growth/ no growth modelling provides an accurate description of the growth limits of *Salmonella* spp. The model developed in this study can help the food industry to support and speed up development or reformulation of safe food recipes. Further work will focus on consolidating parameter distributions, combining the growth and growth/ no growth model with thermal and non-thermal inactivation models in food.

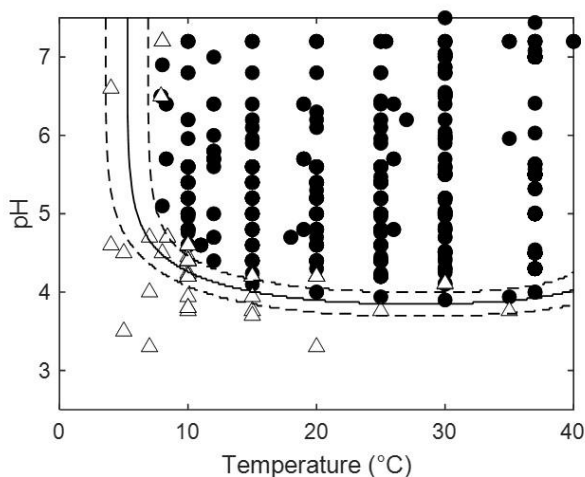
## Supporting info:

### Comparison between observed and predictions (ComBase core data -162 curves)

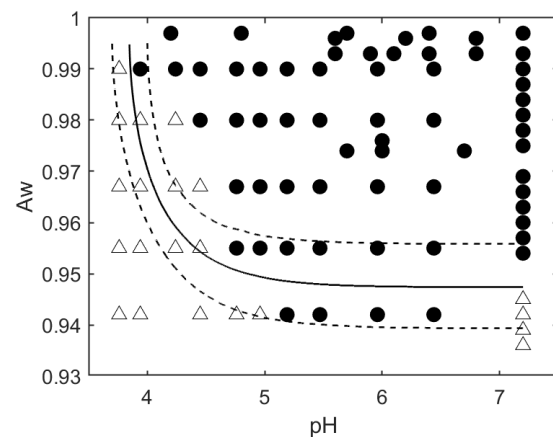


### Growth/ No growth interface- examples of comparison between limits of predictions (quantiles 0.025-0.975) and experimental observations (●: growth, Δ: no growth)

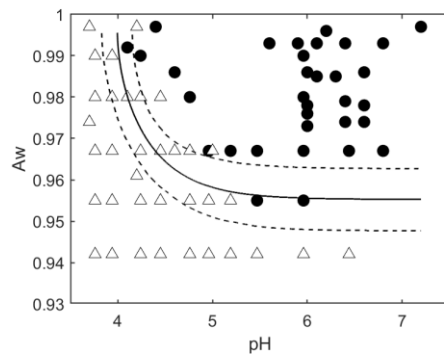
Temperature/ pH ( $a_w > 0.99$ , no lactic acid)



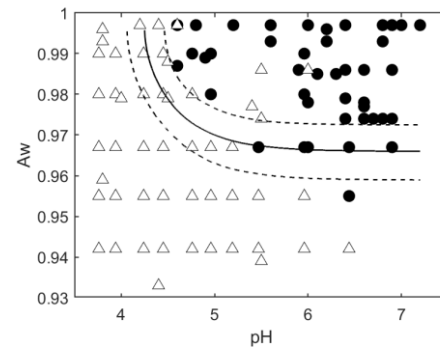
$A_w$ / pH ( $T=25^\circ\text{C}$ , no lactic acid)



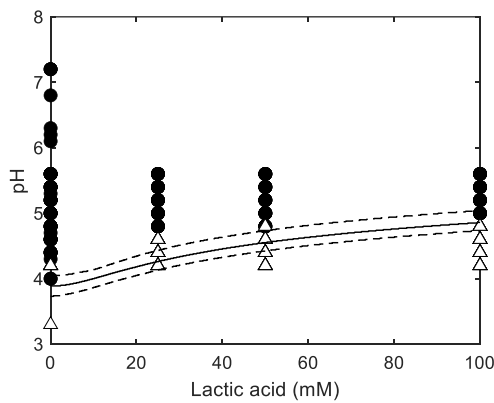
Aw/ pH (T=15°C, no lactic acid)



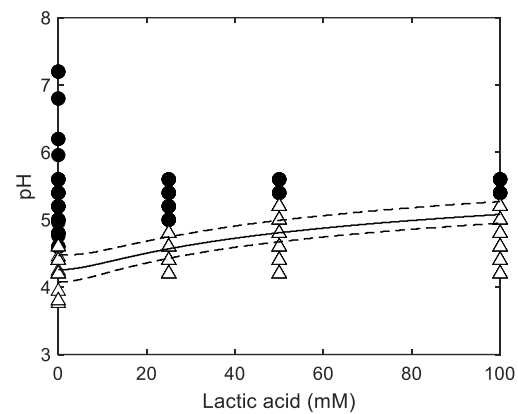
Aw/ pH (T=10°C, no lactic acid)



Lactic acid 20°C (aw 0.997)



Lactic acid 10°C (aw 0.997)



## PP30

### An innovative tool taking account cellular behaviour and phenol content for the quantitative exposure assessment of *Listeria monocytogenes* in smoked salmon

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**Introduction:** Microbiology studies showed that smoked salmon may support the growth of *Listeria monocytogenes*, but with highly variable growth potential. This variability can be linked to several factors, including strain origin, process (e.g., salting and smoking), the physico-chemical characteristics of salmon and background flora. The objective of this study is to develop a probabilistic cell-based model of the growth of *L. monocytogenes* in smoked salmon that incorporates the effects of these sources of variability and takes into account the controlled low prevalence.

**Material and Methods:** The overall model is based on a cardinal model with interaction, which includes the effects of temperature, pH, aw, lactic acid concentration and total phenol content. In addition to these factors, strain variability (n=13), the cellular probability of growth of *L. monocytogenes* (Lm) and the inhibitory effect of lactic acid bacteria (Jameson effect) were taken into account. To validate the model, slices of smoked salmon from several producers were inoculated with low

concentrations of a Lm strain (ADQP 105) at around 1 CFU/g, and the development of the cellular multiparametric growth model was monitored at 8°C/ 10 days. Model predictions were also compared with literature experimental data for  $\mu_{max}$  and cellular probability of growth (cPG).

**Results:** Significant variability in the cellular growth abilities of *L. monocytogenes* is observed according to the physico-chemical properties, including phenol content, concentration of lactic acid bacteria. After 10 days/8°C of storage, the observed experimental concentrations of Lm range between 0 and 6 log<sub>10</sub> CFU/g. For each experiment, the observed concentrations of Lm fall within the range of prediction. The model developed was also successfully validated against literature  $\mu_{max}$  and cPG.

**Conclusion and significance:** The model was implemented in the decision support tool Sym'Previus and will help the food industry to quantify the probability of exceeding threshold concentration of Lm during shelf life.

## PP31

### Validation Of Existing Models for the Description of the Growth of *Listeria monocytogenes* in Primo Sale Cheese

**Erica Tirloni**<sup>1</sup>, Simone Stella<sup>1</sup>, Cristian Bernardi<sup>1</sup>, Viviana Fusi<sup>1</sup>, Per Sand Rosshaug<sup>2</sup>

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Aim of the present study was the validation of two models for *Listeria monocytogenes* growth, previously developed for ricotta, for growth prediction on primo sale cheese. The two models include terms for temperature (Model 1) or temperature and pH (Model 2). Challenge tests with three different brands of primo sale cheese were conducted at various temperatures (4, 6, 8, 12, 16 and 20°C) in triplicate. A dynamic temperature profile was also considered for the validation, with temperatures ranging from 10 to 16°C. During the tests, *L. monocytogenes* count, Lactic Acid Bacteria (LAB) count and pH were determined at each sampling times. Validation of the models included an assessment of the ability to predict maximum specific growth rate using two approaches: bias-factor (Bf) and accuracy

factor (Af), and the acceptable simulation zone (ASZ).  $\mu_{max}$ -values in the challenge tests conducted were, as expected, markedly influenced by the storage temperature with significant increase when the temperature increased from 4 °C to 20 °C. The ability of *L. monocytogenes* to replicate at refrigeration conditions was also confirmed in this study. The variability in pH and LAB counts among the investigated primo sale cheese brands resulted in detectable differences in observed  $\mu_{max}$  values of *L. monocytogenes* in the product. The models could be useful in determining the safe shelf-life of primo sale cheese with the possibility to reformulate the product, aiming to assure the product safety

## PP32

### Risk assessment of marine biotoxin poisoning arising from the consumption of Irish-produced shellfish

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<sup>1</sup>University College Dublin, Dublin, Ireland, <sup>2</sup>Irish Marine Institute, Galway, Ireland

Marine biotoxins, produced by toxic phytoplankton, pose a significant health risk to consumers when they accumulate in shellfish. These naturally occurring toxins can lead to severe foodborne illnesses, particularly when shellfish such as mussels and oysters are consumed. This study develops a comprehensive risk assessment approach for key marine biotoxins, including Diarrhetic Shellfish Poisoning toxins (DSTs) and Paralytic Shellfish Poisoning toxins (PSTs), in shellfish harvested in Ireland. The risk assessment begins with an exposure analysis based on a single eating occasion, considering the natural variability of toxin concentrations among individual shellfish. The model calculates the total toxin load consumed by an individual by summing the toxin content of each shellfish within a serving. Consumption data is derived

from a recent dietary survey on fish and shellfish intake in Ireland. The estimated exposure levels are then compared to the European Union's regulatory limits for marine biotoxin concentrations in shellfish to determine potential health risks. Preliminary findings suggest that regulatory controls, such as the closure of shellfish harvesting areas when toxin levels exceed statutory limits, effectively protect consumers from biotoxin exposure. However, unregulated scenarios, such as casual harvesting of shellfish along shorelines, pose a potential health hazard. Without official monitoring, individuals consuming wild shellfish may unknowingly ingest dangerous levels of marine biotoxins. The study demonstrates the importance of public awareness campaigns, particularly during peak recreational harvesting periods in the summer months.

This work was part funded by the Irish Department of Agriculture, Food and the Marine's Research Funding Programme.

## PP33

### A quantitative microbiological risk assessment model of Shiga toxin-producing *Escherichia coli* contamination for the beef steak supply chain in China

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Shiga toxin-producing *Escherichia coli* (STEC) has become a major public health concern, with beef cattle serving as primary hosts. Evaluating its risks under current consumption patterns is essential for ensuring food safety. Recently, steak has gained increasing popularity among consumers due to its high quality, nutritional benefits, and quicker cooking time compared to traditional Chinese cuisine.

In this study, a QMRA model of STEC for the beef steak supply chain in China was developed to simulate the potential risk and identify key factors critical for risk control. The Monte Carlo simulation method was used for separating the variability and uncertainty of the model's parameters. The stages of industrial processing, distribution, and consumption were considered in the exposure assessment; the prevalence data of STEC from our previous research in Chinese plants was used and combined with a probability distribution method to describe STEC at various stages; the impact of temperature fluctuations during distribution on risk was analysed; and a dose-response model was used to estimate the possible disease risk associated with beef steak consumption.

The model estimated that the mean predicted probability of illness per serving was  $3.17 \times 10^{-7}$ , indicating that there may be 0.03 cases of STEC illness per 100,000. Temperature fluctuations can lead to a 1.6-fold underestimation of the risk when compared to the results obtained under a constant temperature of 4°C during distribution. A sensitivity analysis of relevant parameters in the beef supply chain was conducted, and the results indicate that the cooking process and the initial level of STEC in the hide have a significant impact on controlling the risk of eventual ingestion of STEC-contaminated steaks. The results of the scenario analysis indicate that well-done cooking can reduce the risk by two orders of magnitude. However, given the balance between beef quality and safety, emphasis should be placed on identifying key points and implementing continuous control throughout the beef supply chain.

This study facilitates practitioners to better understand the relationship between pathogenic bacteria and human diseases in the food production process and provides a scientific basis for risk decision-making and food safety governance.

**Keywords:** Shiga toxin-producing *Escherichia coli*, Beef Steak, Predictive Microbiology, Quantitative Microbiological Risk Assessment

## Part B – OMICS and Data Science

PP34

### Quantification of the gastro-surveillance pyramid in The Netherlands: a Bayesian evidence synthesis approach

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<sup>1</sup>rivm, , Netherlands

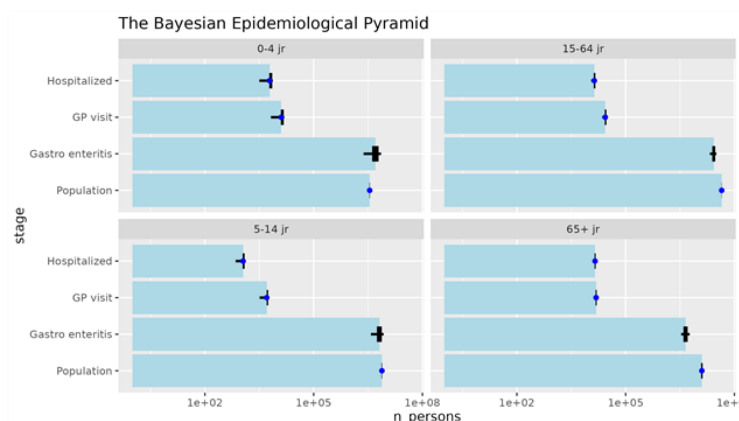
The epidemiology of infectious diseases can be visualised as a pyramid, with on top the diagnosed clinical cases and at the base the fraction of the population infected but unnoticed by the health-care system. Public health surveillance normally only captures diagnosed cases. Information on the total size of the infected population can be obtained by population-based epidemiological studies, including sero-incidence studies. Consequently, each layer of the pyramid is represented by a different type of data, with different study designs. We applied a Bayesian evidence synthesis (BES) modelling approach that, by integrating data from these different studies, was able to quantitatively reconstruct the surveillance pyramid of gastroenteritis (GE) in The Netherlands. In addition, this approach was also able to attribute fractions of GE to different pathogens and age-groups, and capture trends over years.

Data covered the period 2014-2023. Surveillance data consisted of syndromic surveillance at GP and hospital level, and pathogen-specific

laboratory diagnosis . . The cross-sectional population studies included were ESBLAT (2000 persons over two years), ‘family and health’ (2000 persons over two years) and KIzSS (43 child daycare centers over three years).

The BES model jointly estimates a large number of parameters. At the base of the pyramid is the total number of GE events, which is highest for the very young (0-4yr) with 1.4 episodes per year, second highest in children (5-14yr) with 0.9 episodes per year, followed by adults (15-64yr) with 0.6 per year, and lowest in the elderly (>65yr) with 0.4 per year.

The probability of visiting general practitioner (GP) after an GE event is highest for the very young at 0.25% and the elderly at 0.34%. Other age groups are at about 0.1%. Similarly, for the probability of hospitalisation after a GE event, we find 0.3% for the elderly, 0.1% for the very young, and less than 0.05% for the other age groups. The entire pyramid is shown in the Figure. Pathogen attribution was highest for rotavirus and norovirus.



## PP36

### Machine Learning-Based Diagnostic Model for Sarcopenia in Korean Aged 65 and Older Using National Dietary Data

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This study presents the development of an AI-based diagnostic tool for predicting sarcopenia using dietary data from the Korean National Health and Nutrition Examination Survey (KNHANES). The model was built focusing on individuals aged 65 and older, who are at higher risk of sarcopenia. After preprocessing variables such as dietary intake patterns, nutrient consumption, and sarcopenia-related indicators, various machine learning algorithms were applied—including tree-based models, boosting algorithms, and AutoML frameworks such as PyCaret. While boosting models like catboost showed high performance, AutoML approaches facilitated efficient model optimization with minimal manual intervention. Although class imbalance slightly affected overall prediction accuracy, the trained models demonstrated reliable performance. In the

feature importance analysis, age was identified as the most influential factor in predicting sarcopenia, followed by weight and dietary patterns, particularly the frequency of eating out. These findings suggest that both demographic and behavioral dietary variables play a significant role in the early identification of sarcopenia risk. The resulting AI program shows promise as a scalable and interpretable tool for early sarcopenia risk screening among older adults. With continued data expansion and further model refinement, this approach may evolve into a valuable asset for preventive healthcare and nutritional interventions. Ultimately, this study highlights the potential of AI-driven dietary analysis in diagnosing and managing not only sarcopenia but also other nutrition-related health conditions.

## PP37

### Leveraging multi-omics data to predict chicken meat quality and enhance predictive modelling performance using the Multivariate Food Predictor platform

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Technological advancements have enabled the rapid and simultaneous acquisition of large-scale datasets across a range of analytical technologies. Efficient data processing and integration, combined with the extraction of meaningful correlations and the interpretation of complex information, are essential for accurate quality assessment and the support of data-driven decision-making.

This study integrated five analytical methodologies: classical microbiological analysis (total aerobic counts), Fourier-transform infrared spectroscopy (FT-IR), multispectral imaging (MSI), Next Generation Sequencing (NGS), and Gas Chromatography–Mass Spectrometry (GC-MS) for profiling volatile metabolites, utilizing both targeted and untargeted approaches. Data analysis was performed using a new R-based platform called “Multivariate Food Predictor” ([https://skandamis.shinyapps.io/Videometer\\_Predictor/](https://skandamis.shinyapps.io/Videometer_Predictor/)), exploring a range of machine learning algorithms—including Partial Least Squares Regression (PLS-R), PLS-Discriminant Analysis (PLSDA), Support Vector Machines (SVM), Random Forest (RF), and Artificial Neural Networks (ANN)—to assess predictive performance. Model evaluation was based on the root mean squared error (RMSE) and the coefficient of determination ( $R^2$ ), while the weighted regression coefficients of the PLS

models identified the most influential variables contributing to prediction accuracy.

FT-IR, MSI, and GC-MS analyses, individually, demonstrated strong predictive capabilities for estimating microbial populations using the Random Forest Regression algorithm, achieving  $R^2$  values of 0.89, 0.92, and 0.90 with corresponding RMSE values of 0.42, 0.37, and 0.30, respectively. Specific volatile metabolites—2-butanone, acetoin, acetic acid, and 3-methyl-1-butanol—associated with chicken quality exhibited high correlation with FT-IR spectral data. Notably, acetoin levels were accurately predicted using Random Forest ( $R^2 = 0.83$ , RMSE = 7.5 (in the range of 0,00 – 99,5)) and were also linked to variations in storage temperature. Furthermore, specific microbial taxa, including *Acinetobacter*, *Shewanella*, *Pseudomonas*, *Serratia*, and members of the *Vibrionaceae* family, showed associations both with spectral patterns and volatile metabolite profiles.

The cross-validation of findings across multiple analytical techniques, coupled with the utilization of a data-analysis platform that encompasses a consolidated list of the critical machine learning features, friendly to non-expert users, represents a significant advancement in the ongoing development of rapid quality assessment methodologies.

This work has been funded by the EU HORIZON project No. 101136542 FoodGuard

## PP38

### Evaluation of a prototype sensor array for the rapid assessment of Beef Meat Spoilage

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Simple-to-use and direct sensors able to determine the degree of food spoilage are of great interest in the food industry, for real-time monitoring of shelf-life and minimization of food losses.

This study aimed to evaluate a highly sensitive, 'versatile' direct nano-scale spoilage array of sensors for continuous, non-destructive monitoring of beef meat freshness and detection of the extent and type (e.g., acid, alkaline, etc.) of spoilage.

An array of nano-sensors was fabricated using 4 optical sensors, each with a different sensitivity to groups of volatile organic compounds (VOCs). Two of the fabricated sensors were based on Rose Bengal dye, whereas the rest were either based on methylammonium lead tribromide (MAPbBr<sub>3</sub>) and/ or formamidinium lead iodide (MAPbI<sub>3</sub>) nanocrystals (perovskite). Samples (300g) of fresh beef and/or minced-beef meat were received from a local meat company. An array of sensors was incorporated upright into each meat package before being packaged under 80% O<sub>2</sub>:20 % CO<sub>2</sub> (MAP). All meat samples were stored at 4°C until spoilage. At regular time intervals, meat samples were analyzed microbiologically via culture-dependent and

culture-independent (16SrDNA amplicon sequencing) analysis, chemically (VOCs by GC-IMS), and sensorially (CIEL). Each sensor's response to spoilage was monitored visually, by changes in chroma, and through multispectral analysis (VideometerLab).

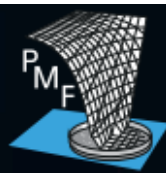
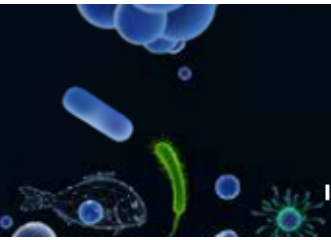
Microbiological, chemical, and sensory assessments agreed with the sensors' visual response to spoilage. A gradual visual change in the color intensity of the optical sensors was observed. Perovskite sensors gradually changed their color (from black to green and from orange to white) during meat storage. The latter color change in the intensity appeared on the 2nd day and gradually increased over the next two days (4-6d) of storage, when a visible change to a darker scale occurred by the 7th day of storage. Similar changes were also observed with the VOCs profiles during meat spoilage as the flavor content of meat samples significantly changed during storage. Moreover, the results of 16SrDNA analysis are indicative of the level of spoilage, as meat freshness was classified into 3 distinct classes.

This study introduces a reliable correlation between the array sensors and meat freshness under food supply chain conditions.

The research project is implemented in the framework of H.F.R.I call "Basic research Financing (Horizontal support of all Sciences)" under the National Recovery and Resilience Plan "Greece 2.0" funded by the European Union – NextGenerationEU (H.F.R.I. Project Number: **17000**).

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## PP39

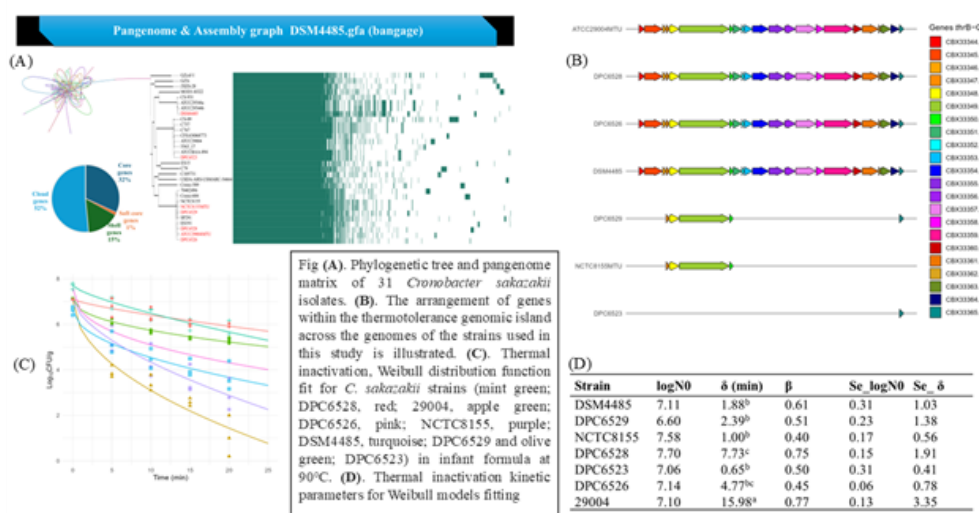
### Thermotolerance of *Cronobacter sakazakii* at elevated temperature in real powdered infant formula devoid of maillard reaction: Inactivation Kinetics, and comparative Genomics

Peter Myintzaw<sup>1</sup>, Fiona Ryan<sup>1</sup>, Aidan Coffey<sup>1</sup>, Michael Callanan<sup>1</sup>

<sup>1</sup>Munster Technological University, Bishop town, Ireland

Despite advancements in food safety standards and control measures, *Cronobacter sakazakii*'s thermotolerance continues to be a serious public health concern, particularly in powdered infant formula (PIF). This study aims to elucidate the strain-specific variations in *C. sakazakii*'s thermotolerance, particularly under the high temperature of 90°C for 20 minutes in a real PIF matrix using distinct strains (n = 7). A significant variation ( $p < 0.05$ ) in the  $\delta$  value (time required for a 1-log<sub>10</sub> reduction from an initial population) amongst the strains was observed when heat treatment data were modelled using the Weibull distribution, indicating substantial strain-specific differences in thermotolerance. No visible colour changes were detected in the PIF under the experimental conditions. In silico multi-locus sequence typing (MLST) analysis based on whole-genome sequencing (WGS) identified the strain set as comprising ST1, ST4, and ST8. WGS from this study, alongside 24 publicly available high-quality genomes' Prokka

annotations unveiled a total of 43 thermotolerance-related genes were present across all genomes. Among these, the presence of hspA, yad\_1, and yad\_2 was enhanced thermotolerance. Additionally, a standalone tblastn search using thermotolerance genomic island (thrB-Q) protein cluster (accession; FR714908.1), unveiled the presence of a complete set of this gene cluster exhibiting enhanced thermotolerance. This study underscores the higher thermotolerance of ST4 strains, emphasising the critical need for stringent safety measures during food production and handling to mitigate the risk posed by these highly resilient strains. The results demonstrate that the thermotolerance variability of *C. sakazakii* were significant and strain-specific differences linked to its genetic content. Such variations in thermotolerance among strains are critical for accurate microbial quantitative risk assessments to mitigate consumer risks associated with PIF.



## PP40

### Creating an innovative digital platform that combines climate models and food safety data to support risk management in response to climate change, using a multi-stakeholder approach

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The AMBROSIA project, titled “Modelling and Communicating Climate Impacts on Food Safety through a Food System Digital Platform,” aims to introduce a comprehensive and systemic approach to food safety risk assessment across Europe’s supply chain in the context of climate change. By leveraging digital technologies, including AI, the project will unfold in three key phases: (i) Developing a holistic risk assessment framework tailored to analyze the impact of climate change on specific food safety hazards, such as *Fusarium* mycotoxins in grains and enteric pathogens like *Salmonella* spp. and *E. coli* in fresh produce. (ii) Integrating spatio-temporal climate model projections with models related to these food safety hazards across Farm-to-Fork, enabling the creation of precise predictive models for different European biogeographical regions. (iii) Building the AMBROSIA digital platform, an innovative tool designed to integrate climate models with food safety data. This platform will provide a user-friendly environment for diverse stakeholders along the Farm-to-Fork chain, facilitating hazard monitoring and supporting mitigation and adaptation strategies.

Acknowledging the vital role of stakeholders in the food value chain, the AMBROSIA project, will adopt a multi-actor approach to establish ecosystems across five Demonstration Regions (DRs) in Europe: Atlantic, Boreal, Continental, and both East and West Mediterranean. Dedicated beneficiaries, known as Demonstration Managers (DMs), will be responsible for ecosystem development to bring together key food system actors, including representatives from the primary sector, the food industry, policymakers, technicians, and food advisors, ensuring a diverse range of expertise and perspectives. The objectives of these five ecosystems are: (i) Identifying key challenges and requirements for the AMBROSIA Platform through co-creative workshops and surveys. (ii) Contributing to the evaluation of the digital platform and its tools via interactive workshops and surveys. (iii) Sharing knowledge and best practices through interviews, covering various stages of food production. (iv) Providing critical feedback on the final version of the AMBROSIA Platform through workshops and surveys.

Acknowledgments: AMBROSIA project GA: 101181300 funded under HORIZON-CL6-2024-FARM2FORK-01-4

## PP41

### Identification of relevant genes for acidic resistance along the pangenome of *S. Typhimurium*: a first step towards acid resistance prediction based on genomic data

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*Salmonella* can adapt and survive under stressful conditions in the food industry due to the great geno- and pheno-typic diversity that characterizes this taxon. One of the challenges these bacteria must face is low pH and acidic conditions. Their adaptability allows them to survive along the production and, not only, the aggressive conditions of the stomach, but also the low pH ambience once they've colonized the enterocytes and have been absorbed by the macrophages.

Our aim is to develop a model, based in a genetic profile, for predicting *Salmonella* ability to resist acidic conditions. This model could help to enhance the reliability of risk estimates for each specific strain depending on their genetic information. To achieve this goal, it's necessary to identify which genes are responsible of different responses.

On the one hand, we have studied the growth fitness of *S. Typhimurium* 14028s in different acidic stress conditions. Based on these results, we defined the optimal combination of growth medium, type of acid, pH and incubation time to perform screening assays to detect the growth fitness cost/benefit associated to the deletion of

single genes under acidic conditions. Then, we tested the response of 3517 isogenic mutants from the Prof. McClelland Single Gene Deletion Library (SGLD)<sup>1</sup> under those conditions. This has allowed us to identify more than 100 genes implied on *Salmonella* acidic resistance.

On the other hand, we generated the *S. Typhimurium* pangenome, from ~1000 genomes representative of the genetic diversity of the serovar and available on the public database Enterobase. We obtained a core genome, constituted by genes that are common in the 98% of the *S. Typhimurium* genomes and an accessory genome, including the genes that characterize individual strains.

We have classified the identified genes from the SGL in the core or the accessory genome. This has allowed us to identify the most suitable genes to constitute the attributes of the predictive model, as they are specific from certain strains.

<sup>1</sup>Porwollik, S., Santiviago, C. A., ... & McClelland, M. (2014). Defined single-gene and multi-gene deletion mutant collections in *Salmonella enterica* sv *Typhimurium*. *PloS one*, 9(7), e99820.

## PP42

### Uncovering Organic Apple Juice Fraud: Analytical Insights from HPLC and FTIR Spectroscopy

Christina Kamarinou<sup>1</sup>, Ismini Patsopoulou<sup>1</sup>, Olga Papadopoulou<sup>1</sup>, Natasa Kapetanakou<sup>1</sup>, Chrysoula Tassou<sup>1</sup>, **Anthoula Argyri<sup>1</sup>**

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Apple juice is often subject to food fraud, underscoring the importance of developing precise techniques to differentiate between organic and non-organic types. Therefore, this study aimed to develop and implement rapid analytical techniques for the discrimination between organic and non-organic apple juice. A total of 30 organic and 29 non-organic apple juice samples were analyzed via Fourier transform infrared (FTIR) spectroscopy, high-performance liquid chromatography (HPLC) for the determination of organic acids (citric, malic, succinic, lactic) and sugars (sucrose, glucose, fructose), Brix and pH measurements. In continuance, the FT-IR spectra collected were background corrected using the standard normal variate transformation. For the HPLC, Brix and pH data, no pretreatment was applied. The database was partitioned into a training (70%) and a validation (30%) dataset. Subsequently, the normalized data were mean centered and were

used to build partial least squares regression (PLS-R) models for the 2-step classification of the apple juice samples in the organic or the non-organic group class. The FT-IR models were evaluated both with and without feature selection. Regarding the PLS–FTIR classification models for the validation dataset, the results have shown an overall classification accuracy of 65.45% without feature selection and an improved accuracy of 85.45% after feature selection. In both cases, the models showed higher accuracy in predicting the non-organic samples, with only 1 non-organic sample being classified as organic after feature selection. The classification accuracy of the PLS–HPLC/Brix/pH and PLS–HPLC models reached values of 100% for both cases, without feature selection. This research highlighted the efficiency of analytical methods to ensure the quality and integrity of apple juice in both the organic and conventional markets and prevent fraud.

**Acknowledgement:** This work was funded by the European Union, under grant agreement No 101083579 - THEROS project. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for them.

## PP43

### Sustainability Optimization for Secure Food Systems using the potential of data exploitation technologies and AI: the Athens use case

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<sup>1</sup>Yiotis Anonimos Emporiki & Viomixaniki Etaireia, Athens, Greece, <sup>2</sup>SmartAgroHub S.A., Athens, Greece, <sup>3</sup>Hellenic Agricultural Organisation DIMITRA, Athens, Greece, <sup>4</sup>Agricultural University of Athens, Athens, Greece

SOSFood project aims to develop an AI-driven food sustainability assessment system using interoperable data. It will develop predictive tools for better decision-making in the food supply chain. These solutions will be validated through case studies in three different scenarios (regional – Galicia, metropolitan – Athens, and national – Lithuania). In each use case, two different approaches for predictive analysis on time series data will be explored: (1) Single response prediction, aimed at inferring a model from the set of training data for extrapolating future trends on a single output variable of interest; (2) Multi response prediction, where the goal is to jointly predict the future trends of multiple output variables, assuming that these variables show multiple dependencies among them in the form of correlations.

To assess the viability of a potential use case, it is important to first analyze the objectives, available data, and key processes that can benefit from predictive analysis. In the Athens use case, key parameters of baby food production are analyzed, with a focus on optimizing the selection of raw materials that contribute most significantly to final product costs. The primary goal is to prevent price

increases that directly affect consumers, while ensuring microbiological safety and compliance with regulatory standards through predictive analysis.

The approach combines cost-efficiency with quality assurance, using a methodology that includes:

- i) Data collection from price trends and private databases on biological and chemical hazards in cereals and milk powder (e.g., mycotoxins, pesticides, chlorates);
- ii) Integration of external factors, such as seasonal price fluctuations, market demand, environmental conditions (e.g., droughts, floods), and broader social influences (e.g., wars, pandemics);
- iii) Predictive modeling to forecast both price and quality trends; and
- iv) Development of a decision-support tool that balances cost, safety, and quality metrics for purchasing decisions.

The expected outcomes include i) More strategic, data-informed procurement of raw materials; ii) Cost savings without sacrificing product quality; and iii) Improved product safety and consistency, driven by predictive, real-time quality control mechanisms.

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## PP44

### Depicting the prevalence and assessment of colonization of *Salmonella* in fresh produce

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The microbial quality of fresh-cut produce and pathogen survival and growth on fresh-cut produce is influenced by several interdependent factors including product type, minimal processing operations and packaging. Moreover, during production of fresh-cut produce, cumulative mild processing steps are employed, to control microbiological growth. Thus, pathogens on plant surfaces are already stressed and stress may be increased during the multiple mild processing steps, potentially leading to very hardy bacteria geared towards enhanced survival. The aim of this research project is the quantitative assessment of the salmonellosis risk associated with the consumption of fresh produce (e.g., leafy salads). To this direction, the expression of genes

(in vitro and in situ) associated with colonization, biofilm formation, stress, pathogenicity and quorum sensing (QS) necessary to colonize and persist in the fresh produce will be monitored. To better depict this phenomenon, the factors affecting the colonization including pre-contamination conditions, pathogen – plant interactions and microbial interactions will be assessed. Finally, the reconstruction of gene regulatory network of pathogens during biofilm formation and communication via QS, will be the fundamental research target point for identifying the underlying molecular mechanism to achieve a greater insight on the impact on human health after consumption of contaminated fresh produce with *Salmonella*.

**Acknowledge:** The research project is implemented in the framework of H.F.R.I call “3rd Call for H.F.R.I.’s Research Projects to Support Faculty Members & Researchers” (H.F.R.I. Project Number: 23271).

## PP45

### Impact of Environmental and Operational Factors on Kimchi Maturation in a Pilot-Scale Cold Storage Room: Validation of KFRI RAS - A Cloud-Based Analytical Platform for Data-Driven Food Research

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Modern data-driven research requires powerful analytical tools to integrate disparate datasets across disciplines, facilitate statistical modeling, and support machine learning-based predictions. To this end, the KFRI Research Data Analytics Supporter (RAS), an officially copyrighted data-driven analytics program, was developed as a deliverable of the Cloud-based Food Data Analytics Platform research project. The platform allows researchers to collect, cleanse, manage, and pre-process research data in a unified environment, streamlining the entire data analysis workflow.

To validate the program's performance, we used kimchi quality data from the retail environment accessible within the institute's internal data repository. The entire data preprocessing pipeline, including data cleaning, management, and transformation, was executed exclusively within KFRI RAS without the need for external software. The platform's computational accuracy was cross-validated against MATLAB, a commercial numerical computing software, and Colab, a cloud-based open-source environment, to ensure the same numerical output across all systems.

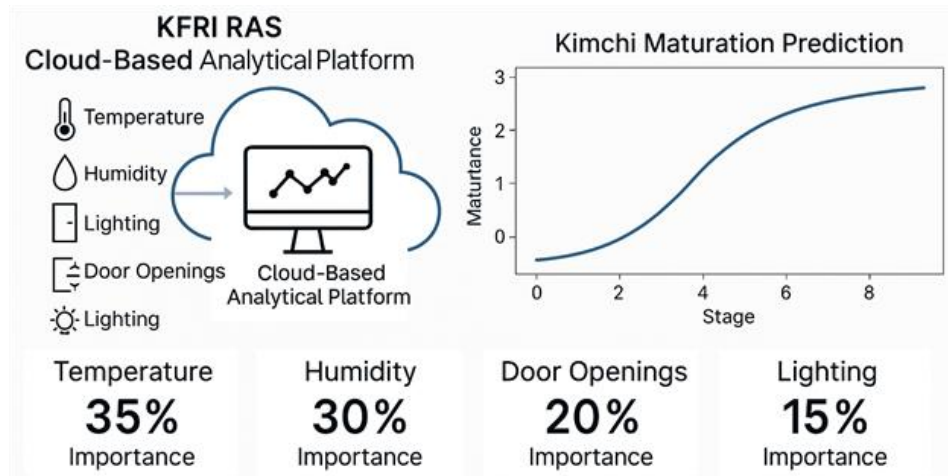
Using a random forest model implemented in KFRI RAS to predict the kimchi ripeness index (0:

less fermented to 10: fully fermented), we obtained a root mean square error (RMSE) of 0.52 and an  $R^2$  of 0.89. Feature importance analysis of the other included features identified temperature and humidity as the main influencing factors, with door openings and shelf position also contributing significantly. Additional predictive improvements were also observed for lighting intensity and airflow, demonstrating the platform's ability to handle multi-factor data.

The study found that KFRI RAS performs statistical analysis and predictive modeling comparable to commercial software, while providing an integrated cloud-based solution tailored specifically for research institutions. While the study is primarily focused on food quality monitoring, future applications will leverage the platform's advanced analytics capabilities to expand applications into food microbiology, safety assessment, and dietary data analysis. The results of this study confirm the potential of KFRI RAS to foster innovation in food science and safety research as a scalable, research-driven alternative to data-driven research analytics tools.

#### Acknowledgements:

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## PP46

### Creating a Digital Shadow: Leveraging Data Science for Decision-Making in the Food Sector

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**Introduction:** In response to evolving consumer demands and industry guidelines, the food sector is increasingly adopting data-driven decision-making tools to speed up time to market while maintaining food safety. This abstract explores the development of a data-driven model that serves as a digital counterpart to microbial predictive models.

**Methodology:** The Corbion Microbiology and Data Science groups have developed predictive models to forecast the outgrowth of *Clostridium perfringens* after cooling cooked meat. Additionally, a digital shadow has been created to identify optimal ranges for antimicrobial agents, aiming to reduce outgrowth to less than one log, in line with USDA-FSIS stabilization Appendix B guidelines for food safety.

**Results:** The surrogate model, which acts as a digital replica of the food system, consists of an equation with second-order polynomial and interaction terms, retaining only the significant terms. This model allows for the visualization of the effects of various inputs on the response, enabling the identification and selection of

optimal ranges for the most important variables. By interactively adjusting the levels of inputs, the impact of different variables on the response and achievement a targeted theoretical minimum for outgrowth can be visualized. The model ultimately suggests appropriate antimicrobials dosage based on specific conditions and requirements. This approach enables internal and external stakeholders to optimize the formulation and processing conditions without the need for extensive physical trials, saving both time and resources.

**Conclusions and Relevance:** The development of this data-driven model represents a significant advancement in the food sector's ability to ensure product safety and quality. By leveraging predictive and surrogate models, informed decisions can be made about antimicrobial usage and optimized conditions to control pathogen growth. This approach will not only assist the food processors to align with regulatory guidelines but also address consumer demands for safer and higher-quality food products.

## PP47

### Assessing foodborne outbreak risk in Chinese households: A national survey analysis of pork handling practices

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**Introduction:** Foodborne diseases pose a significant global health threat. Despite a significant number of consumers suffering from foodborne diseases, attention has barely been paid to domestic food handling practices. The best way to quantify the risk is to develop a Quantitative Microbiological Risk Assessment (QMRA) model. However, QMRA models require a substantial amount of data, which significantly limits the application of this model, as obtaining all the needed data is challenging.

**Methodology:** This study aimed to assess the risk of foodborne outbreaks associated with household food handling practices and identify the consumer groups that are at higher risk for foodborne outbreaks in China. A national survey of household pork handling practices was conducted in China. The risk of foodborne outbreaks stemming from consumers' improper handling practices was assessed utilizing the Weighted Harmonic Outbreak Prevention Index (WHOPi). This index assigned varying weights to each practice based on the likelihood of certain behaviors causing foodborne diseases and categorized these practices into three levels of

good hygienic practice (GHP): low (WHOPi ≤ 0.35), medium (WHOPi: 0.36–0.70), and high (WHOPi > 0.70). Logistic regression analyses were used to identify groups at higher risk for foodborne outbreaks.

**Results:** A total of 2730 households from seven provinces in mainland China were analyzed. Nearly two-thirds of households (64.8 %) didn't have a separate cutting board for raw meat, and 44.9 % claimed to use the same board to handle ready-to-eats afterward. Based on WHOPi, only 0.4 % of households demonstrated a high level of GHP, while 55.4 % showed a low level of GHP. The primary non-conforming stage was separation practices, followed by cooking and purchasing practices. Logistic regression indicated more attention is required for those living in southern and rural areas, working in non-public institutions, possessing lower levels of education, and being single or widowed.

**Conclusion and relevance:** The household food handling practices of Chinese consumers involve serious food safety risks. Education programs and interventions concerning safe handling practices in households are urgently needed.

## PP48

### FluoPath: Development of fluorescent biomarkers in two foodborne pathogens to better predict the impact of food processing on their survival and virulence in dairy products

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The FluoPath project is funded by the French National Research Agency for the period April 2024-March 2028 and accredited by 2 competitive clusters: Vitagora and Valorial. This project brings together 7 partners, including 4 academic research laboratories, 2 agro-industrial technical institutes and the French dairy interbranch.

Guaranteeing food safety and reducing economic losses are two challenges facing the dairy industry. The main objective of the FluoPath project is to identify new biomarkers (promoters that induce the expression of genes of interest) coupled to a fluorescent biosensor (transcriptional fusion) to gain new insights into the physiological state of two foodborne pathogens (*Listeria monocytogenes*<sup>1</sup> and *Bacillus cereus*<sup>2</sup>) in dairy environments (milk, diluted model cheese and, if possible, solid model cheese) in relation to the impact of technological perturbations on bacterial resistance, growth and virulence. Ultimately, the knowledge acquired during the project, combined with existing scientific literature, will contribute to enhancing predictive models for microbiological exposure assessment in dairy

products. Predictive models will be built, based on (i) single-cell modeling approaches for post-stress probability of growth, lag and generation of individual bacterial cells and (ii) the correlation of fluorescence signal with survival, virulence, entry in growth phase, initiation of toxin production and entry into sporulation.

This project, which involves multiple partners, will generate disparate types of data (such as cultivability, transcriptomics, flow cytometry<sup>3</sup>) with a view to developing predictive models. Consequently, particular attention is being paid to data management and sharing, with standardization playing a pivotal role. This is motivated by the necessity for each partner to be able to apply the same experimental protocols and statistical analyses to ensure data quality. The experimental protocols are shared and validated between at least two partners before being rolled out to the entire consortium. The statistical analyses employed to substantiate the conclusions will be meticulously designed to facilitate the implementation of suitable tests, encompassing the assessment of data normality and homoscedasticity.

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## PP49

### Predicting stress tolerance phenotype of *Listeria monocytogenes* using genome (omic) data and machine learning algorithms

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*Listeria monocytogenes* constitutes an inconvenient hurdle for the dairy industry. It is of high importance to be able to associate the genotype with phenotype as variability exists between strains regarding their response to different stresses. In this way we could identify genetic elements that are linked with high risk strains. Such genetic elements might also be used to predict the stress tolerance phenotype. The objective of this work was to combine genome data with machine learning algorithms to create a predictive model that could accurately predict the stress tolerance phenotype. To this end, the available whole genome sequencing and food-related stress data of 166 *L. monocytogenes* strains (Hingston et al., 2017) were used. To predict the different stress tolerance phenotypes (cold, acid, salt, and

desiccation), we trained different machine learning models (Random Forest, Support Vector Machine, and XGBoost) on four different genomic representations (153 strains): presence/absence of gene families, the 8 nucleotide long sub-sequences of their DNA (8-mers), the 9 nucleotide long sub-sequences of their DNA (9-mers), and the 11 nucleotide long sub-sequences of their DNA (11-mers). The models had high Pearson Correlation coefficients and were validated on a separate dataset of 13 strains. The results suggest that omics and machine learning can be integrated in QMRA studies by developing genomic-based predictive models which in turn can be used to accurately predict the bacterial phenotype of a foodborne pathogen supporting more efficient decisions about risk.

#### Reference

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## PP50

### Database Development for the Integration of Kinetic and Probabilistic Models

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Deterministic and stochastic modelling are often treated as separate research approaches to predictive microbiology. The deterministic approach is the primary tool at cell population level, especially for spoilage organisms. In contrast, stochastic modelling is critical to assess microbial food safety at low cell concentrations. This study aims to integrate the two methods, relying on kinetic as well as probabilistic and growth / no-growth data, for either spoilage or pathogenic organisms, to improve the accuracy of microbial risk assessments in food safety.

The first step is to develop a database that accommodates data of both kinds of responses. This database is designed to be compatible with ComBase ([www.combase.cc](http://www.combase.cc)), a database and

predictor of kinetic kind. The key difference between the two is the extended interpretation of the response variable. While the kinetic approach considers the log of the bacterial cell concentration as an elementary piece of information, from which growth / death rates can be derived, the analogy for this, in case of stochastic approach, is the growth / no-growth binary variable, from which the probability of growth can be derived. In both cases the variable can be temporal, lending itself to dynamic modelling.

Exploiting the potential of binary data and establishing connections with kinetic data will enable users to increase the efficacy of predictive modelling and therefore improve the microbial safety of food.

## PP50\_a

### Whole-Genome Characterization of *Listeria monocytogenes* to Inform Risk Models in Food Environments

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Biofilm formation significantly enhances bacterial survival and persistence in various environments, posing major challenges to public health, food safety, and environmental management. In this study, 50 *Listeria monocytogenes* isolates were analyzed, originating from environments related to the food industry—including the fishery and meat sectors—as well as from clinical isolates. The main objective of this work was to characterize the genetic determinants involved in biofilm development to generate genomic data applicable to Quantitative Microbial Risk Assessment (QMRA) models.

A structured Whole Genome Sequencing (WGS) pipeline was implemented, comprising data preprocessing, genome assembly and

annotation, and the identification of genes associated with biofilm formation (such as adhesion factors, extracellular matrix production, quorum sensing mechanisms, and antimicrobial resistance). Additionally, plasmid content analysis was performed to identify mobile genetic elements that may contribute to persistence and virulence in complex environments.

This approach provides a solid foundation for integrating genomic information into QMRA models, improving the understanding of biofilm-associated microbial risk of listeriosis and supporting the development of effective mitigation strategies in both clinical and food production contexts.

#### Keywords:

bacterial genomics, biofilm, resistance genes, virulence genes, microbial risk assessment, predictive microbiology

## Part C – Modelling Food Microbiome

PP51

### Predictive Modeling of Salmonella spp. Inactivation in Peanut Butter at Different pH and Aw and Process Establishment

**Alessandra Regina Da Silva**<sup>1</sup>, Izael Gressoni Junior<sup>2</sup>, Pedro Xavier Rodriguez Massaguer<sup>1</sup>, Pilar Rodriguez de Massaguer<sup>1</sup>

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The thermal resistance of *Salmonella* spp. in low-moisture foods, such as peanut butter, poses significant food safety challenges, mainly because in these environmental, heat resistance is increased. Processors must establish combinations of time and temperature that reduce food safety hazards during distribution and storage. This study aimed to determine the thermal inactivation parameters ( $D^*$  and  $z^*$  values) of *Salmonella* spp. in 2 peanut butter formulations: 1. pH 6.49 and water activity ( $a_w$ ) = 0.278 and 2. pH 6.06 and  $a_w$ =0.564; and develop predictive models for its thermal inactivation. Heat resistance assays were conducted using a mix of *Salmonella enterica* serotype Choleraesuis and *Salmonella enterica* serotype E, both isolated from peanut butter samples. The bacterial suspension was standardized to 109 CFU/mL and inoculated into peanut butter samples formulations 1 and 2, followed by thermal treatments at 71°C, 85°C, 90°C, and 93°C, conducted in duplicate. Survival counts were determined, and data were fitted to the Weibull model to estimate the scale ( $\alpha$ ) and shape ( $\beta$ ) parameters and for establish the time to 7 log reductions. After that, the linearization of curves ( $D^*$  and  $z^*$  values) was performed according to Pflug (1999).

Results indicated non-linear thermal inactivation behavior at all tested temperatures, with higher temperatures leading to more rapid bacterial reductions. The shape parameter ( $\beta$ ) was consistently  $<1$ , indicating upward concavity of survival curves ( $R^2>0.93$ ). At 93°C, complete *Salmonella* elimination was achieved within 12 minutes in integral peanut butter 1 ( $a_w$  = 0.278) and within 8 minutes in peanut butter 2 ( $a_w$  = 0.564). The  $D^*$  values decreased with increasing temperature, and, the  $z^*$  value for peanut butter 1 was 7.31°C and for peanut butter 2, 7.13°C. The process validation confirmed that thermal treatments of 93°C for 40min and 93°C for 8min (obtained by Weibull modelling), for peanuts 1 and 2, respectively, were effectively achieved 7-log reduction of *Salmonella* spp., ensuring product safety.

These findings highlight the importance of predictive modeling in optimizing thermal processing conditions for peanut butter pasteurization, contributing to microbiological food safety. The Weibull model proved to be a reliable approach for characterizing microbial inactivation kinetics in low-moisture food matrices.

## PP52

### Temperature-Driven Growth Dynamics of *Staphylococcus aureus* in Artisanal cheese: Insights from Predictive Microbial Modeling

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Ensuring the microbiological safety of traditional dairy products demands a deep understanding of pathogen behavior under varying environmental conditions. This study investigated the growth dynamics of *Staphylococcus aureus* CECT 976 in Moroccan Jben, a soft goat's milk cheese produced without starter culture, and stored at 8°C, 20°C, and 37°C. Using lab-scale cheese prepared following artisanal methods and inoculated with *S. aureus*, we estimated key kinetic parameters including initial and maximum microbial concentrations (SA<sub>0</sub>, SA<sub>max</sub>) and maximum growth rate ( $\mu_{\max}$  SA) through Lotka-Volterra and Jameson-effect models, accounting for the inhibitory influence ( $a_{21}$ ) of indigenous lactic acid bacteria (LAB).

The results revealed striking temperature-dependent differences in the growth dynamics of *S. aureus* and LAB. At 8°C, *S. aureus* exhibited lower initial and maximum concentrations alongside slower growth rates ( $\mu_{\max}$  SA: 1.054 days<sup>-1</sup> and 1.089 days<sup>-1</sup> for Lotka-Volterra and

Jameson-effect models, respectively). In contrast, at 37°C, *S. aureus* displayed a sharp increase in growth rate (14.97 days<sup>-1</sup> and 22.24 days<sup>-1</sup>) and maximum population levels. The non-significance of the inhibitory parameter ( $a_{21}$ ) across temperatures underscored the Jameson-effect model's parsimony. Consequently, square-root secondary models for the maximum growth rates of *S. aureus* and LAB were derived from Jameson-effect estimates and validated against independent growth data obtained at 30°C.

These findings offer valuable insights into the temperature-driven growth behavior of *S. aureus* in Jben cheese, offering a robust foundation for developing predictive models to enhance the microbial safety of traditional dairy products while preserving their cultural authenticity.

**Keywords:** Jben cheese, *Staphylococcus aureus*, microbial modeling, Lotka-Volterra model, Jameson-effect model, lactic acid bacteria.

## PP53

### Evaluation of quality and safety of sea bream (*Sparus aurata*) using classic and rapid detection methods

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**Introduction:** Sea bream is a highly perishable food, requiring prompt and reliable microbial quality assessment. Advanced detection techniques combined with machine learning offer a promising method for real-time visualization and analysis of food quality, improving safety and shelf-life monitoring.

**Materials and Methods:** Farmed whole gutted fish were packed in modified atmosphere packaging (45% CO<sub>2</sub> / 35% N<sub>2</sub> / 20% O<sub>2</sub>) and stored at 0, 4, 8, and 12 °C. Microbiological counts and pH were monitored throughout storage, and sensory assessments were conducted. Additionally, Fourier transform infrared (FTIR), and multispectral imaging (MSI) were applied as rapid, non-destructive techniques to estimate the microbiological status of samples from the fish's flesh and skin. For spectral data analysis, 70% of each dataset was used for training and 30% for testing the models. Partial Least Squares Regression (PLS-R), Support Vector Machine Regression (SVM-R), and K-Nearest Neighbors Regression (KNN-R) models were implemented to predict microbiological counts. In contrast, Partial Least Squares Discriminant Analysis (PLS-DA), SVM, and KNN models were used for qualitative

prediction, correlating spectral data with sensory evaluations to classify samples as fresh or spoiled.

**Results:** Results showed that the initial microbial population of sea bream was 3,1 log CFU/g (TVC) and the dominant microorganisms were *Pseudomonas* spp. and H<sub>2</sub>S-producing bacteria. At all temperatures, bacteria increased rapidly and achieved high populations by the end of storage (5,3 – 7,8 log CFU/g). Based on sensory scoring, the shelf life of the samples was 18, 12, 6, and 5 days after storage at 0, 4, 8, and 12 °C, respectively. pH values were similar during storage and ranged between 5,8 and 6,3 for all storage temperatures. Regarding the predictive models, the regression ones did not yield satisfactory results compared to the classification models. Specifically, for qualitative prediction, the SVM model provided the best results for skin and flesh samples, achieving 50% to 90% accuracy for all datasets.

**Discussion and Relevance:** The results of the current study are promising since they highlight the potential of FTIR and MSI, combined with machine learning models, for the rapid detection of the microbial quality and shelf life of fish.

**Acknowledgments:** This work has been funded by the project FOODGUARD 'Microbiome applications and technological hubs as solutions to minimize food loss and waste' – FOODGUARD, Horizon-IA 101136542.

## PP54

### Intelligent food packaging in couple with Food Spoilage and Shelf-life Prediction Module for seafood distribution control

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According to FAO, 14% of the food produced globally is estimated to be lost, while another 17% is wasted, causing severe environmental impact, challenging food security, and leading to increased food costs. Bacterial contamination of seafood shortens its shelf-life, leading to losses, and may also cause food safety issues. The packaging process protects food products from bacterial contamination thereby preserving their freshness and structural integrity, increasing shelf-life and enabling long-term storage. Recent advancements of active/smart packaging technology focus on maintaining the quality and enhancing the shelf-life of food products by incorporating functional agents (e.g. antimicrobial) into the packaging material. Intelligent packaging technology monitors (with precision via on-package sensors) extrinsic food parameters (e.g. temperature) that help to ensure freshness, quality, safety, and security along the value chain. QuiPack project focuses on advanced bio-intelligent coatings/packaging materials, that meet both customer and market requirements, supported by AI and IoT devices (that form in essence an intelligent food value chain), as well as decision support systems

optimized with regard to food quality and safety, are key elements towards the next step in enhanced food safety and quality monitoring and reporting, leading to minimization of costs and waste. QuiPack will also develop and subsequently validate predictive models to achieve high efficiency in terms of population determination of spoilage microorganisms. For selected spoilage microorganisms, their spoilage domain will be identified, and shelf-life can therefore be predicted, evaluated and subsequently stored on the database of the Project. To provide an integrated system for product quality and safety monitoring that allows interfacing with diverse user groups and devices (e.g., smartphones, logistic platforms, etc.), a cloud-based data-handling platform will be developed. QuiPack's Food Spoilage and Shelf-life Prediction Module will leverage predictive microbiology to implement cloud user interfaces accurately predicting the growth of spoilage microorganisms and shelf life. The outputs of the models will be directly translated by the module into shelf-life for the stakeholder to provide them with an estimation of the expiration date/shelf-life of seafood.

**Acknowledgements:** This work was funded by Project PRIMA 2023 "QuiPack". Food value chain intelligence and integrative design for the development and implementation of innovative food packaging according to bioeconomic

sustainability criteria. PCI2024- 153530 financed by MCIU/AEI/10.13039/501100011033 / UE

## PP55

### A review of quantitative data for modelling transconjugation of Antibiotic Resistance Genes in the food chain

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Antibiotic resistance (AR) has arisen as a global public health concern over the last few years, due to the increasing incidence of AR human pathogens. The food chain plays a critical role in AR dissemination, due to the use of antibiotics in animal production and the impact on zoonotic diseases. To incorporate the role of antibiotic resistance genes (ARGs) transmission into quantitative risk assessment frameworks, it is essential to gather data on transconjugation rates of ARGs in food microbiota and pathogens, and to integrate this knowledge into predictive microbiology approaches.

This study aimed to collect and analyze quantitative data regarding the ARGs transfer rates, identifying the most common donor and recipient species, and examining the main conjugation factors influencing ARG dissemination in food-related microbial ecosystems.

An extensive literature review was conducted using databases such as PubMed (NIH), ScienceDirect, ResearchGate, and Web of Science. The search strategy was based on keywords such as "antibiotic resistance genes", "horizontal gene transfer", "food microbiota", and "foodborne pathogens". The results were extracted based on primary experimental studies

reporting quantitative data on ARG transfer rates under food simulation conditions.

Most collected conjugational rates were reported from filter mating assays involving donors from species of lactobacilli and enterococci isolated from ready-to-eat foods or fermented foods. Recipients included the same genera as well as foodborne pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica*. In general, ARGs studied were associated with resistance to tetracyclines, erythromycin, and vancomycin. Reported conjugation rates (on a scale 0-1) ranged from 1 to 10<sup>-10</sup>, with foodborne pathogens such as *L. monocytogenes* and *Y. enterocolitica* exhibiting rates between 10<sup>-6</sup> and 10<sup>-10</sup>. Temperature conditions commonly found in food environments (i.e., 10-37°C) were shown to support ARGs transfer.

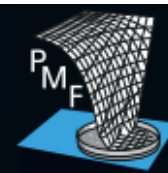
The quantitative data collected in this study provide a foundation for incorporating ARG dynamics into probabilistic microbial risk assessments. Specifically, these values can be integrated as a genetic layer within predictive approaches for microbial interactions, enabling a better representation of microbial dynamics and spatiotemporal variations of ARGs patterns along the food chain as well as their impact on foodborne pathogen risk.

#### Acknowledgements

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# 13th ICPMF

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## PP56

### Natto-InsPired bioPreservation of plaNt food matrix (NIPPON) - A project from the French Ferments du Futur program

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The NIPPON project focuses on the microbiological safety of plant-based foods by drawing inspiration from Asian fermentation traditions, particularly natto. Natto is a Japanese superfood fermented by *Bacillus subtilis*, rich in vitamin K2, poly-γ-glutamic acid, and nattokinase, with remarkable properties for inhibiting foodborne pathogens.

The goal of NIPPON is to understand how *Bacillus subtilis* prevents the growth of pathogenic bacteria in plant-based foods. By modeling microbial interactions, identifying key

genes involved in biopreservation, and exploring the inhibitory capacities of various non-lactic bacterial species, the project aims to develop new food preservation systems.

These innovative biopreservation systems will meet the growing demand for safe, natural food products without the use of chemical preservatives. By leveraging the natural preservation mechanisms found in natto, NIPPON aspires to enhance the safety of plant-based foods on an industrial scale, offering healthier alternatives to consumers.

## PP57

### Prediction of strain's evolution by thermal inactivation: random walk following between- and within-strain variabilities

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Thermal inactivation is one of the most general methods for controlling food microbial risk. The magnitude of thermal inactivation tends to be weakened based on the hurdle theory by demands for natural tastes or flavors. However, if heating does not inactivate all bacteria, selection by thermal inactivation could result in the evolution of heat resistance. This study proposes the simulation methodology of evolutions of bacterial heat resistance using between- and within-strain variabilities. The reduction behaviors of 50 *Campylobacter jejuni* strains heated at 55°C were described with Weibull models using the most probable curve method. The multivariate normal distributions described the between and the within-strain variabilities in delta and power parameters. Here, the simulation assumed the within-strain variability as the potential of the parameter change from one strain (power to evolve) and the between-strain variability as the distribution that heat resistances of all strains follow (gravity of species). The simulations of 10000 replicates, initial counts of 4 log CFU for each inactivation, 100 heating times for one simulation, and 0-, 2-,

4-, and 6-log reduction heating. The parameters of surviving strains after heating were decided with the Monte Carlo simulation, and the parameter's evolution was estimated with random walk using the Metropolis-Hastings algorithm. After a hundred selections by thermal inactivation, the surviving ratio was derived as 99.3% with 2-log, 52.0% with 4-log, and 4.73% with 6-log reduction heating from 10-thousand replicates of simulations. It was simulated that the selections by the thermal inactivation raised the heat resistance of *C. jejuni*. This simulation estimated the strain variabilities after the multiple heating and population growth. The evolutionary behaviors of heat resistance caused by mild thermal inactivation could be important for microbial risk in foods or the quantitative microbial risk assessment. This study proposed the simulation method for heat-resistance evolution using the random walk with between- and within-strain variabilities. The developed evolutionary simulation could provide critical information for food processing repeating thermal inactivation.

## PP58

### Food safety: Research into the antimicrobial activity of an S90 lactic acid strain against harmful bacteria

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#### Introduction:

Lactic acid bacteria are generally selected as starters for their technological properties. In the food industry, they are used in the fermentation of foods, in the production of flavours and in the preservation of foods through the production of acids and bacteriocins .

#### Materiel and method:

The aim of this work is to demonstrate the inhibitory effect of strain *Lactococcus lactis* S90 on pathogenic bacteria by the production of acid and bacteriocin was investigated using three methods: the Fleming and al (1985) method, the Barefoot (1983) method and the disc method.

#### Results:

These techniques showed that harmful bacteria were inhibited by the acid and two bacteriocins produced by strain S90, one of which was a protein that acted on *E.coli* and the other a glycoprotein or lipoprotein that acted on *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*. These substances produced by S90 have bacteriostatic activity on undesirable bacteria.

#### Conclusion

*Lactococcus lactis* strain S90 can be used in the food industry as a food preservative.

**Keywords:** Starter, preservation, bacteriocin, bacteria, lactic, undesirable

## PP59

### Microbiome applications and technological hubs as solutions to minimize food loss and waste - FOODGUARD

**George - John Nychas**<sup>1</sup>

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FOODGUARD aims to develop and demonstrate co-created solutions that will support innovations & advances based on microbiome, microbial activities & technology hubs to address food, health, economic and environmental challenges. The envisioned approach consists of a framework of toolsets & methodologies to provide sustainable solutions in food processing, packaging & across the food value chain to address food shelf-life increase & waste reduction in a holistic manner. The proposed solutions aim to (a) extend food shelf life with novel packaging/ biopreservation i.e. use of protective cultures/synthetic microbial consortia, recyclable films with natural antimicrobials or protective cultures; (b) monitor food quality/safety/shelf life with microbial indicators/molecular biomarkers used in smart packaging (TTIs, smart printed tags, non-invasive sensors) & (c) accurately predict food shelf life & improve traceability using predictive models, AI/ML, Internet of Things & tools like QR, NFR etc.; FOODGUARD toolbox components will be

extensively evaluated in real life settings through four pilot demonstrations in 4 different countries with involvement of all relevant actors while covering diverse requirements and different food products. FOODGUARD outcomes target i) to minimize food loss and waste by shelf life extension and prediction, ii) to help the food industry to implement these preservation solutions as an alternative to chemical preservatives, iii) to deploy responsive policy for implementing these approaches as well as to engage consumers educated via tools/platforms, effectively improving awareness & trust in the food sector to (iv) increase traceability, providing real-time supply transparency that will improve the uptake of data-driven innovations in food systems, optimize resource efficiency (reducing food waste from farm to fork) (v) manage increased complexity in agri-food production & supply chain process, make it easier for consumers to adopt a healthy & safe food diet.

## PP60

### A Probabilistic Assessment of Minimum Inhibitory Concentration (Mic) Using Extreme Value Theory and Single-Cell Analysis

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Antibiotic resistance in pathogenic bacteria is a growing global concern, posing significant threats to food safety and human health due to the widespread and often inappropriate use of antibiotics. Conventional methods for determining the Minimum Inhibitory Concentration (MIC) typically rely on serial two-fold dilutions and focus on bacterial populations as a whole, overlooking concentration intervals between standard dilutions and individual cell behavior. However, even within genetically identical bacterial populations, phenotypic heterogeneity can lead to varied single-cell responses to antibiotics under different environmental conditions. This study investigates the behavior of individual *Salmonella enterica* cells exposed to antibiotics and assesses how this variability influences the MIC. For this, a modified agar dilution method was employed incorporating both serial two-fold dilutions and intermediate antibiotic concentrations. Four distinct *Salmonella enterica* strains were surface-plated on agar media containing varying ampicillin concentrations, with inoculum levels ranging

from  $10^2$  to  $10^4$  CFU/mL. With the assumption that each bacterial colony originates from a single cell, differences in colony formation revealed significant variability in individual cell MIC. Fitting the data to various probability distributions showed that the ampicillin MIC of *Salmonella enterica* single cells follows a normal distribution for all tested strains with different parameters for each strain. Monte Carlo simulations further illustrated the impact of inoculum size on MIC values of *Salmonella* populations. Population MIC values were further analyzed using the extreme value theory. By linking parameters of the normal and Gumbel distributions, a framework to calculate MIC while accounting for population size and acceptable risk was established. This probabilistic approach provides new insights into MIC estimation, offering an understanding of antimicrobial resistance. It also serves as a valuable tool for developing targeted antimicrobial management strategies, improving the accuracy of MIC measurements, and addressing the challenges posed by antibiotic-resistant bacteria in clinical and food safety contexts.

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## PP61

### Mathematical Modeling of the Development of *E. Coli* and *Staphylococcus Sp* (Coagulase Positive) Inoculated in Meat Treated with Essential and Vegetable Oils.

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Meat has always been one of the most appreciated foods in Argentina. Technological developments in its processing and preservation have allowed for greater selectivity in searching for the highest quality. Thus, to ensure its preservation, it is necessary to employ various methods that prevent microbial growth, prolonging its shelf life. In this regard, essential and vegetable oils are presented as a possible alternative. One of the most notable antimicrobials is oregano oil (OE), while olive oil (O) is characterized by its stability and antioxidant properties.

The objective of this work was to mathematically model the development of *E. coli* ATCC 25922 and *Staphylococcus sp.* (coagulase positive) inoculated separately in meat samples, with and without the addition of a mixture of oils, stored under refrigeration.

The oils were obtained by different methods, and the corresponding MIC was determined from their mixture. The microorganisms were then inoculated separately on bovine buttock samples, dividing them into two batches: 1)

sprayed with the MIC (T) and 2) untreated (C). Subsequently, they were stored at different refrigeration temperatures (0, 4, and 8°C), and the corresponding counts were performed at different times on selective media. The results were analyzed using the Gompertz's and linear regression models, and the parameters derived from the models were calculated. Finally, the activation energies ( $E_a$ ) were determined by applying the Arrhenius model.

The MIC for *Staph.* was 2.50% V/V, while for *E. coli* it was 1.25% V/V. A good fit of the experimental data to the models was observed. In all cases, the final counts of the T samples were lower than those of the C samples, showing the greatest differences at 8°C in the case of those inoculated with *Staph.* (1.04 log CFU.cm<sup>-2</sup>), which, in turn, in the case of the C samples, reached the highest growth rate (0.43 log CFU.cm<sup>-2</sup>. days<sup>-1</sup>). The latter microorganism also presented the highest  $E_a$  values (271.02 kJ. mol<sup>-1</sup>). From these results it can be concluded that the joint application of these agents can be effective as inhibitors of bacterial growth.

## PP62

### Development of a strawberry freshness prediction model with electronic nose

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#### Introduction

Strawberries are perishable agricultural products that can deteriorate due to gray mold and biochemical degradation during storage. Strawberries produce undesirable aromas due to chemical reactions and microbe growth during deterioration. An electronic nose is likely to identify the changes in the aroma. An electronic nose based on a gas chromatography(FGC-FID) system has also the potential to assess food quality (freshness) in the supply chain. This research aims to analyze the quality indexes and VOCs profile changes in strawberries at constant temperatures during a certain time of storage.

#### Material and methods

The correlation between volatile compounds and microbial concentrations was studied and prediction models of ten extracted aroma compounds were established by partial least squares regression (PLSR). A multivariate partial least squares (PLS) analysis based model was developed to predict the storage time, total aerobic bacterial count and total yeast and mold count of strawberry. The PLS algorithm is based on linear regression methods. Y is the matrix built

with the electronic nose measurement (gas sensors) and X is the matrix containing the productive values (AC and YM).

#### Results

Prediction model of total yeast and mold count by volatile compounds could represent the actual data with minimum latent variables (9 and 10 variables) and could perform with satisfied results such as yeast and mold at 4°C (R<sup>2</sup> = 0.77 and RMSE = 0.30), at 10°C (R<sup>2</sup> = 0.96 and RMSE = 0.22) and at 15°C (R<sup>2</sup> = 0.94 and RMSE = 0.21). The prediction models (PLSR-AC) with 8 latent variables could show good performance which predict the strawberry quality stored at 4°C with performance values (R<sup>2</sup> = 0.72 and RMSE = 0.35), at 10°C (R<sup>2</sup> = 0.84 and RMSE = 0.33) and at 15°C (R<sup>2</sup> = 0.88 and RMSE = 0.28).

#### Conclusion

Electronic nose has been proven and practically can be integrated with various non-destructive techniques for monitoring the food quality and microbial count in food products.

## PP63

### NextFoodPack project: Integrated design and evaluation of new-generation packaging to protect perishable food products

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The ambitious goal of France's anti-waste law No. 2020-105 is to drastically reduce the use of single use plastics by 2040. While this target can be met for rigid plastic packaging through mechanical recycling, this recycling method is not yet sufficiently mature for flexible plastic packaging, which accounts for 45 % of all food packaging. In this case, the only way to achieve a reduction is to replace flexible plastic films with more sustainable solutions, such as paper-based materials, monomaterial films, and bio-based compostable blends.

In addition, the new European Packaging Packaging Waste Regulation (PPWR), adopted on April 24, 2024, sets a target of 55% packaging recycling rate by 2030. A key sector is modified atmosphere packaging (MAP) for perishable foods, such as meat and cheese, which represents a \$15.9 billion market growing at a rate of 4.8% per year. MAP technology requires high gas barrier properties, necessitating major innovations. Current alternatives remain insufficient, and more in-depth assessments of health safety are needed, particularly for managing microbiological and toxicological risks.

The main objectives of the NextFoodPack project are:

- to design and optimize new flexible packaging able to meet the multiple requirements of MAP in terms of barrier properties, chemical and microbiological safety and whose end-of-life could be managed by developing innovative processes combining recycling and decontamination.
- to generate fundamental knowledge about formulation and generation of degradation products during processing and use, enabling a solid evaluation of toxicological safety using biotests able to evaluate cocktail effects, and original methods for rapid toxicological assessment.
- to create a software tool to manage microbiological risks and predict food shelf life in MAP.
- to develop an optimization tool integrating technical, environmental, health, safety, and social criteria to support the food and packaging industry make decisions to propose tailored and sustainable MAP for each specific use case.

#### Acknowledgement

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## PP64

### Machine learning for fish spoilage classification: A feasibility study using spectroscopic sensors

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**Introduction:** Rapid and objective assessment of fish spoilage is crucial for maintaining food quality. Traditional methods, such as microbial and sensory analysis, are time-intensive and subjective, highlighting the need for automated, non-invasive alternatives. Machine learning (ML) applied to spectroscopic data offers a promising approach for real-time freshness evaluation. This study investigates the feasibility of ML-based classification for fish spoilage detection using Fourier-transform infrared spectroscopy (FTIR) and multispectral imaging (MSI) data.

**Methodology:** Spectral data of FTIR (n=178), benchtop-MSI (n=195) and portable-MSI (n=198) were collected from sea bream fillet samples stored at 0, 4, 8, and 12 °C under aerobic conditions. Samples were classified based on sensory assessment (scores 1.5-3). Specifically, classes were determined by averaging the scores of the examined sensory attributes (aroma, texture, color). Samples with scores between 1.5 and 2.5 were classified as “fresh”, while those above 2.5 were labeled as “spoiled”. Eight distinct ML classification models were evaluated to classify samples into the two classes. For each dataset, stratified sampling was applied so that 80% was used for training and 20% for testing the models. Model performance was based on

accuracy, Cohen’s kappa, ROC-AUC, precision, recall and F1-score.

**Results:** The ML models demonstrated adequate potential for spoilage detection, with accuracy ranging from 60.7% to 91.2%, depending on the sensor and model. The FTIR dataset outperformed the other sensors, with Partial Least Squares Discriminant Analysis (PLS-DA) achieving the highest accuracy (91.2%) and ROC-AUC of 0.85. For benchtop-MSI, the Ridge model achieved an accuracy of 80%, with ROC-AUC equal to 0.73, suggesting a reliable classification of samples. For portable-MSI, PLS-DA performed best, reaching 75% accuracy and an ROC-AUC of 0.80. However, some models showed moderate accuracy. These results underscore the importance of sensor selection and ML model optimization in food quality monitoring.

**Conclusions and Relevance:** This study confirms the feasibility of ML-based, non-invasive spoilage detection using spectroscopic data. The results highlight the potential for real-time food quality monitoring while emphasizing the importance of selecting models tailored to specific datasets. These findings contribute to the development of automated quality control solutions, supporting food safety and waste reduction across the food industry.

**Acknowledgments:** This work has been funded by the project FOODGUARD ‘Microbiome applications and technological hubs as solutions to minimize food loss and waste’ – FOODGUARD, Horizon-IA 101136542.

## PP65

### Egg freshness prediction and monitoring using a sensor tag based smart distribution system

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#### Introduction

Eggs are stored and transported in a variety of environments from the farm to the consumer, and it is critical to maintain egg quality during the distribution processes. In this study, a technology for predicting the quality of egg according to temperature was presented using a smart distribution system based on RF sensors to monitor the quality change of egg during distribution in real time.

#### Material and methods

The smart distribution system is a convergence of food quality prediction dynamic model technology and IoT-based food distribution environment monitoring technology. By attaching RF sensor tags that measure temperature to food packaging boxes, information is delivered by predicting in real time what effect the food will have on the quality of the food when it is exposed to a temperature higher than the standard during the distribution process. To predict the quality (Haugh unit, Albumen Index and Yolk Index) of egg, we developed predictive models using kinetic for the

primary model and Polynomial for the secondary model.

#### Results

Quality assessment results showed that HU, AI, and YI tended to decrease with increasing storage duration and temperature, with more rapid degradation observed under variable temperature conditions. As a result of predicting the quality change of egg under real temperature conditions, Af and Bf showed an accuracy close to 1 under both fluctuating temperature conditions (Af = 1.01, Bf = 0.99 and RMSE = 0.04). And, as a result of determining the quality grade by linking the developed egg quality prediction model with the smart distribution system, it showed an accuracy of over 95%.

#### Conclusion

Food quality predictions using temperature histories based on RF sensors can help consumers make reasonable sales decisions. The findings of this study provide optimized storage conditions to maintain egg freshness in a dynamic retail environment.

## PP65\_A

### Development of Predictive Models for Microbiological Quality Assessment of Whole Sea Bream (*Sparus aurata*)

**Fotoula Schoina**<sup>1</sup>, Stamatina Xenou<sup>1</sup>, Angeliki Doukaki<sup>1</sup>, Olga Papadopoulou<sup>2</sup>, Chrysoula Tassou<sup>2</sup>, Panagiotis Skandamis<sup>3</sup>, George-John Nychas<sup>1</sup>, Nikos Chorianopoulos<sup>1</sup>

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**Introduction:** Each year, over 10 million tons of seafood products are spoiled or damaged during transportation or storage. As a result, real-time monitoring of seafood freshness is of great importance. The aim of this study was to monitor the spoilage of whole ungutted sea breams and develop models that can predict their microbiological quality.

**Methodology:** Whole ungutted sea breams were preserved at 4 different temperatures (0, 4, 8 & 12 °C) under modified atmosphere conditions (40% CO<sub>2</sub>, 20% O<sub>2</sub> & 40% N<sub>2</sub>). During the study, microbiological analysis, pH measurements and sensory evaluation were performed, while spectral data from three different parts of the fish (flesh, skin & gills) were obtained at the same time intervals using two multispectral imaging instruments (Benchtop-MSI & Portable-MSI), NIR spectroscopy and Fourier transform infrared spectroscopy (FT-IR). Then the spectral data were processed and used in PLS-Regression, KNN-Regression and SVM-Regression models to estimate the microbial counts of sea breams.

**Results:** Microbiological results showed that the initial population of the sea breams ranged from 4.2 to 4.5 log CFU/g, while *Pseudomonas* and H<sub>2</sub>S producing bacteria were among the dominant spoilage microorganisms. Shelf-life, determined by sensory evaluation, of whole sea bream stored at 0, 4, 8 & 12 °C was 17, 16, 6 and 4 days, respectively. The performance of the models was evaluated in terms of root mean square error (RMSE) and R<sup>2</sup>. According to the developed models, most of them had satisfactory predictions, with Portable-MSI-based models for the gills being the best ones. More specifically, for the Benchtop-MSI models R<sup>2</sup> values ranged from 0.172 to 0.331, while for the Portable-MSI models R<sup>2</sup> ranged from 0.259 to 0.645, with the KNN model showing the best performance with R<sup>2</sup> = 0.645 and RMSE = 0.712.

**Conclusions and relevance:** The collected information from this study is promising, while further investigation is needed in terms of prediction of microbiological quality and safety.

This work has been funded by the project FOODGUARD, Horizon-IA 101136542.

## PP74

### AI-assisted Quality Assessment of Strawberries Using Deep Learning Models: A tool for food waste reduction applications

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Recent advancements in deep learning (DL) have paved the way for advanced methodologies to evaluate and classify agricultural produce. This study focuses on employing DL algorithms to classify the visual appearance of strawberries using their images to enable food quality assessment and food waste management. The analysis was conducted using MATLAB software, where three DL architectures—GoogleNet, ResNet-50, and Inception-v3—were utilized for classification tasks. The dataset was divided into two subsets, with 80% of the images used for training the models and 20% reserved for testing. This split ensured a robust evaluation of the models' performance and generalization capabilities. The models demonstrated exceptional performance, with all achieving classification accuracies exceeding 96.1% for appearance prediction. Among these, the Inception-v3 model emerged as the top performer, attaining a remarkable accuracy of

98.1% during the testing phase. Based on its high performance, the Inception-v3 algorithm was selected as the classification algorithm for developing a dedicated software application for strawberry appearance classification. The software provides a reliable, automated solution for assessing the visual quality of strawberries, streamlining the classification process for industrial applications. This system benefits both suppliers and customers by ensuring consistent quality standards and providing suitable tools to enhance shelf-life assessment and food waste reduction. In addition, the findings highlight the transformative potential of deep learning in enhancing quality control processes within the food industry. This approach could be efficiently integrated into digital food traceability and logistics systems to automatically monitor vegetable quality in real time thus supporting timing and reliable decision-making processes along the food supply chain.

Keywords : Deep Learning, Quality Assessment, Classification, Strawberry

## Part D – Back to Future Roots of PMF

PP66

### From Digestion to Bioactivity: In Silico Characterization of Osteopontin-Derived Peptides from Human, Bovine, and Caprine Milk

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A multifunctional glycoprotein found in human and animal milk, osteopontin (OPN) plays a role for gut development, cellular communication, and immunological regulation. Bioactive peptides with potential health advantages are released during OPN digestion. The intention of this research were to examine the in silico digestion of OPN from human, bovine, and caprine milk and describe the functional characteristics of the OPN-derived peptides.

In order to simulate the gastrointestinal digestion process for both premature and normal term infants, the digestion simulations were carried out using computational enzymatic hydrolysis models (PeptideCutter and BIOPEP). Using a variety of bioinformatics tools, the OPN-derived peptide profiles were investigated of their potential for antimicrobial (CAMPR4), allergenic (AllerTop v2.1), toxicity (ToxinPred2), cell penetration possibilities (C2Pred), and bioactivities (BIOPEP).

Two different digestion models (premature and normal-term) were used to digest the proteins. The three OPN sources (*Homo sapiens* (hOPN), *Bos taurus* (bOPN), and *Capra hircus* (cOPN)), which are extensively employed in the infant feeding and formula industries, showed notable

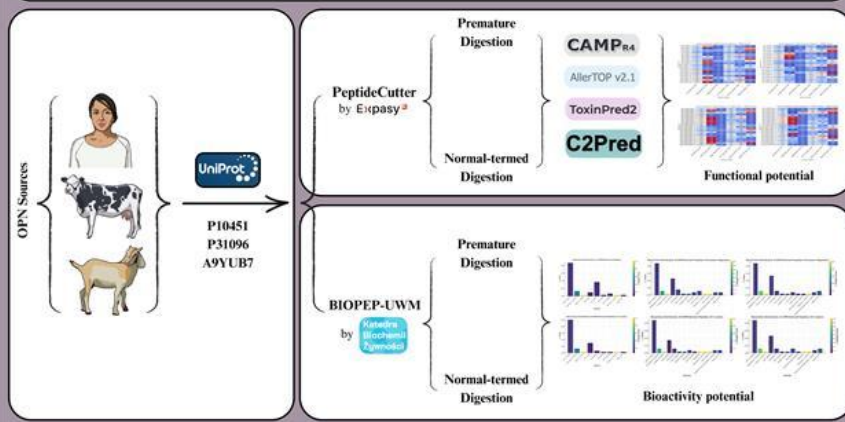
differences in peptide production. 43.59% of hOPN, 23.53% of bOPN, and 37.14% of cOPN were projected to have the capacity to generate AMP-effective peptides out of all the peptides generated in the analyses carried out utilizing the CAMPR4 tool. The production potential of hOPN was 33.33% for allergenic peptides and 48.72% for toxin-effective peptides, compared to 35.29%-50% for bOPN and 34.29%-54.29% for cOPN. The ability of hOPN to generate peptides with ACE Inhibitor and Dipeptidyl Peptidase-IV (DPP-IV) Inhibitor actions makes it stand out in the investigations carried out utilizing BIOPEP. bOPN and cOPN-derived peptides have a notable antioxidative activity, according to the predictions.

These results underline the crucial role of OPN-derived peptides in nutrition and host defense and provide guidance on their functional potential. Peptide bioactivity is influenced by the milk source, according to the comparative analysis of human, bovine, and caprine milk OPNs. This could have implications for pharmaceutical applications, functional food creation, and infant nutrition. Validation of these computational predictions demands further experimental analysis.

#### Acknowledgements

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### From Digestion to Bioactivity: In Silico Characterization of Osteopontin-Derived Peptides from Human, Bovine, and Caprine Milk



## PP67

### Standardization of the Cardinal Values Determination and Use to Predict Microbial Growth: The development of the ISO 23691 standard to strengthen Food Safety

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**Introduction:** Among available predictive microbiology (PM) models, cardinal models have been widely used to predict microbial growth in foods. While several methodologies to determine cardinal values (CV) were previously published, international standardization was missing. This lack of a consensual reference document including a standard protocol and clear quality criteria hampered the development of robust and reproducible PM-based studies to justify control measures.

**Purpose:** The ISO 23691 standard aims to provide harmonized guidance for CV determination and their use to predict microbial growth considering temperature, pH, aw and different inhibitors.

**Methods:** Developed within ISO/TC 34/SC 9/WG 19, the ISO 23691 standard gathered experts from Academia, Industry, Food Safety Agencies and Food Safety Authorities from several countries in Europe and the US. The methodology involves a standardized step-wise approach: (i) the assessment of  $\mu_{max}$  in broth in different conditions (ii) the determination of the CV, (iii) the execution of challenge tests to estimate the food correction factor and provide validation datasets, (iv) the determination of the quality criteria for the CV associated with pass / fail criteria, and (v) the simulation of growth under

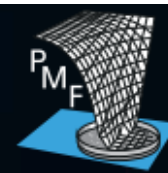
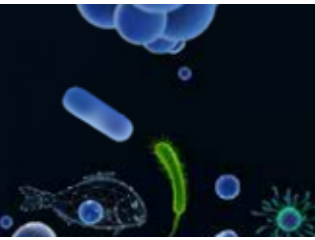
static or dynamic scenarios using deterministic or stochastic approaches.

**Results:** ISO 23691 provides detailed procedures for experimental design, model fitting, and validation, allowing its standardized application in different laboratories. The proposed methodology, with its quality criteria, guides the users of the standard when poor quality datasets are obtained, thus preventing the use of parameters associated with high uncertainty. The standard also highlights the importance of considering the food correction factor and the validation step before proceeding with the predictions. Applications span from shelf-life validation, to product formulation and intermediate storage duration assessment during operations. The timely publication in July 2025 will soon facilitate the ISO 23691 use by Food Business Operators in Europe to demonstrate their compliance with *L. monocytogenes* food safety criteria.

**Significance:** The significance lies in the international consensus achieved among experts from academia, industry, and regulatory bodies, making this the first globally agreed-upon protocol in the field, improving transparency, reproducibility, and

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acceptance of predictive microbiology-  
based results.



## PP68

### The impact of weak organic acid salts and water activity on the growth rate of lactic acid bacteria

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#### Introduction

Lactic acid bacteria (LAB) are well known foodspoilers with significant food waste impact, poses a persistent challenge in the food industry. Understanding the predictive microbiology of LAB is crucial for devising effective control strategies. In this study, we assessed the effects of propionate, lactate, acetate and the water activity on the growth dynamics of LAB in broth. Growth curves were fitted and secondary model was selected to evaluate the effects of organic acids and water activity on specific growth rates.

#### Material and Methods

A gamma model was developed for predicting the growth of three well known LAB spoilers in food, *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and *Lactobacillus curvatus*. Strains were inoculated in broth at 20°C, pH of 6.0 and different levels of salt (0-9%w/w) or sodium propionate or sodium lactate or sodium acetate (0-4.5%w/w). Collection of the growth data was done using absorbance measurements. Values of maximum specific growth rate were calculated by fitting the data with the modified Gompertz equation. Secondary model (Hill equation) was selected to calculate the normalized dose response curves of salt (water

activity) and organic acids. Maximum growth rate ( $\mu_{\text{opt}}$ ) was determined by the average of all control treatments in the different experiments.

#### Results

The growth parameters were estimated for every strain from experimental data fitting at 20°C and pH of 6.

The  $\mu_{\text{opt}}$  results vary between 0.11 and 0.21 ln cfu/h which indicate a great spread between the lactic acid bacteria where *L. mesenteroides* has the lowest growth rate. The minimum water activity varies between 0.929-0.953. Sodium acetate has MIC values between 4.1-5.6%, while sodium lactate ranges from 5.6% to over 7.5%. These values indicate the concentrations needed to inhibit microbial growth. *L. curvatus* is most sensitive for sodium acetate (4.1%) and shows hardly inhibition with sodium lactate (>7.5%). Sodium propionate shows equal performance for all strains (4.4-4.7%).

#### Conclusion

Using the developed model, various combinations may be tested virtually before doing expensive and time consuming inoculation studies on real product.

## PP69

### Modelling of heating profile, particle dynamics, and microbial lethality in radiofrequency treatment of vegetable and fish purees

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Radiofrequency (RF) is a thermal processing technology that uses electromagnetic energy to heat more rapidly and uniformly than conventional methods. The aim was to develop and validate a model for predicting RF pasteurization and sterilization of vegetable and fish puree (V&F puree) and to simulate the associated microbial lethality.

Commercial finite-element software COMSOL Multiphysics was used to predict the temperature profiles and distributions through a PTFE container filled with the puree. Particle tracing was added to the model to calculate the temperature and F-value ( $D_{70^{\circ}\text{C}}=-0.83$ ;  $zT=9.1^{\circ}\text{C}$  and  $D_{120^{\circ}\text{C}}=-0.78$ ;  $zT=10.2^{\circ}\text{C}$ ) for 100 particles during the whole interval of the heating process. Model validation involved RF-treatments on V&F puree inoculated with *Pedococcus acidilactici* (pasteurization) and *Geobacillus stearothermophilus* spores (sterilization). Temperature was recorded using an optic fibre sensor and thermographic camera.

The developed model accurately predicted the temperature of puree during RF heating and subsequent cooling. In general, for the three RF-pasteurization treatments (max. core temperature of 65, 70 and 80°C) the RMSE values were low (0.065, 0.039 and 0.034, respectively).

However, greater deviation from the simulated values was observed (RMSE=0.179) in RF-sterilization (120°C). Simulated and experimental temperature distributions indicated consistently higher temperatures at the container's bottom compared to near the lid. Particle tracing showed that the particles had different heating rates. For RF-pasteurization, F-values at 65, 70 and 80°C were  $3.6\pm0.4$ ,  $9.7\pm0.5$ ,  $128.1\pm25.1$  min, respectively, which were within the predicted F-values ranges. Experimental F-values at 70 and 80°C were close to the median of the predicted F-values of the 100 particles (6.09 min and 150.33 min, respectively). While in sterilization, experimental F-value ( $7.6\pm0.5$  min) was above the maximum predicted value (3.8min). Regarding the challenge test, *P. acidilactici* was inactivated  $2.7\pm0.2$  and  $>9$  logs at 65 and 70°C, respectively. In RF-sterilization, *G. stearothermophilus* spores showed less than 1 log reduction. These experimental values were within the predicted inactivation ranges by the temperature profiles of the 100 particles.

The developed model can be useful to define the RF-treatment conditions to ensure food processing efficacy and food safety, highlighting its potential as an alternative to conventional thermal treatments.

**Keywords:** Radiofrequency, Sterilization, Pasteurization, microbial lethality

## PP70

### Data-Driven Decision Support Predictive Tools: The Industry Perspective

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Keywords: food safety, validated models, preservation, pathogen control, innovation, AI-powered model

**Introduction:** Driven by consumers' demands, new trends, and specific guidelines, the food industry is constantly seeking preservative solutions targeting product (re) formulation, such as increasing meat pH, lowering salt (sodium) content, vegan and plant-based new recipes, use of clean-label and natural preservatives. These trends, however, represent a challenge for the industry, which needs to perform several challenge tests to (re) formulate their products without any indication of potential success. Using validated and up-to-date predictive models implemented in user-friendly tools can strongly help food processors meet specific safety & shelf-life requirements.

**Methodology:** This study aims to present the industry perspective on the benefits of developing and using fit-for-purpose AI-powered predictive model tools through (i) model development (ii) model implementation in user-friendly interfaces, and (iii) model maintenance with cycles of updates based on new trends & customer feedback. These steps are demonstrated, in this study, for Corbion Listeria Control Model (CLCM®), a data-driven tool aiming to predict the *Listeria monocytogenes* outgrowth in different food categories.

**Results:** A global approach and cross-functional interactions (IT, Data Science, Predictive Microbiology, Business, Marketing) are crucial to tackling the multidisciplinary complexity of developing, implementing, and maintaining predictive model tools. Once validated predicted models are implemented in a user-friendly interface, it is important to keep them updated including the addition of internal and external specifically designed challenge test data, customer feedback, market demands, and model improvement/refitting. Validated & up-to-date predictive models can help the food industry to overcome challenges when (re) formulating products to ensure food safety and increase speed-to-market.

**Conclusions and relevance:** Data-driven decision support predictive tools assist food processors in assessing potential food safety hazards for new product formulations. These tools significantly reduce R&D costs and time, increase success rates in challenge tests, and expedite innovation to market. To enhance their credibility and trustworthiness, validated predictive models must be supported by regular updates, which require cross-functional collaboration.

## PP71

### Development of a decision support tool based on predictive models for the evaluation of alternative additives against foodborne pathogens and spoilage bacteria in cooked meats.

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The aim of this project is to assist food industry in assessing the effectiveness of alternative additives when designing new products, allowing manufacturers to predict microbial growth or inhibition of certain microorganisms at different conditions. This tool helps ensure compliance with regulations and provides a user-friendly interface for decision-making in industrial settings for the meat industry.

Recent developments for this tool involve the study of additives such as INBAC ACN/NA able to inhibit the growth *Listeria monocytogenes* and lactic acid bacteria in a cooked meat products or the study of a nitrite and nitrate free additive (INBAC NF) developed and evaluated against non-proteolytic *Clostridium botulinum* and *Clostridium aldicarnis*, a psychrotrophic microorganism isolated from spoiled cooked meat.

To obtain the predictive models on which this decision support tool is based, tests were done in cooked meat products that were produced with the addition of different doses of INBAC ACN/NA (0.1 to 0.5%) or INBAC NF (0,025 to 0,3%). Cooked meat products were inoculated with the target microorganisms in separated

experiments. The meat was then sliced, vacuum packed and stored for up to 120 days under different temperatures (5°C-25°C). Analysis of samples to estimate the growth of the target microorganism was done at several time intervals and curves were adjusted using the Barany model (Barany, J. and Roberts, T.A. 1994). Secondary and Tertiary models were obtained for a range of doses of the additives and conservation temperatures.

A new development is currently in course aiming the inhibition of spore forming bacteria in meat products that suffer a sublethal inactivation treatment and therefore can survive and compromise the stability of this products at room temperature. One of the parameters of this model is the treatment temperature that can contribute to sensitise the bacterial spores and made them more susceptible to the natural additive.

This decision support tool is providing food producers a safe way to replace synthetic preservatives obtaining cooked meat products with longer shelf life and better consumer acceptance.

This project has been funded by Centro para el Desarrollo Tecnológico Industrial (CDTI), Ministerio de Ciencia, Innovación y Universidades (España).

## PP72

### Mathematical evaluation of bioactive compounds recovery from *Auxenochlorella pyrenoidosa*: the effect of non-thermal technologies

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*Auxenochlorella pyrenoidosa* is a microalga that stands out due to its high protein content and quality, while containing significant amounts of natural pigments, such as chlorophylls. However, due to the rigidity of the *A. pyrenoidosa* cell wall, effective cell disruption methods are required to recover these intracellular compounds, while avoiding their degradation. This can be achieved by applying nonthermal technologies for cell disruption, such as pulsed electric fields (PEF) and high-pressure homogenization (HPH).

*A. pyrenoidosa* suspensions (2.5% w/w) were treated to various PEF (4.6 kV/cm, 53.8-179.2 kJ/kg) and HPH (400-800 bar, 4 passes) conditions. For chlorophyll extraction, PEF-treated biomass was incubated in ethanol solutions (75-95% v/v) at 30, 45, and 60 °C for up to 6 h. For protein extraction, HPH-treated biomass was incubated in water at 20, 30, and 40 °C for up to 24 h. The evolution of each extraction process was mathematically evaluated with one overall mathematical model as a function of all pretreatment and incubation parameters. To validate the developed mathematical models, independent experiments of extraction at different experimental conditions were carried out. To incorporate parameter variability, the

Monte Carlo simulation was appropriately performed on the developed mathematical models.

The developed mathematical models describing the recovery of chlorophylls from PEF-treated biomass and proteins from HPH-treated biomass presented satisfactory goodness of fit with coefficients of determination ( $R^2$ ) higher than 0.9 and root mean squared errors (RMSE) lower than 1.0 mg chlorophylls/g and 13.0 mg proteins/g, respectively. Additionally, the relative error (RE%) between the validation experimental values and predicted values did not exceed 17% for the chlorophyll extraction model and 10% for the protein extraction model.

This study demonstrates the industrial potential of *A. pyrenoidosa* as a sustainable source of natural bioactive compounds, emphasizing the role of nonthermal technologies in enhancing extraction efficiency. The optimized PEF and HPH treatments enabled selective and high-yield recovery of chlorophylls and proteins, while the developed mathematical models are valuable industrial tools for accurate prediction of extraction yield and estimation of extraction efficiency for different process parameter combinations.

## PP73

### A Cold Chain Data Based Tool for Shelf Life Determination and Dynamic Assessment

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Shelf life determination is a critical factor in ensuring both consumer safety and product quality. Considering that systematic shelf life studies are labor intensive and time-consuming, the accelerated shelf life testing (ASLT) methodology, based on product exposure to controlled elevated conditions, offers a viable and challenging solution for food industry, allowing for faster shelf life predictions. On the other hand, when referring to the actual cold chain, in practice, significant deviations from specified conditions often occur, that may determine the risk potential, the shelf life and final quality of chilled products processed and packed under Good Manufacturing Practices and Good Hygiene Practices. Thus, temperature variability has to be taken into account for cold chain control and any logistics management system that aims on product quality optimization at the consumer's end.

The Cold Chain Predictor (CCP) software, developed and implemented within the European project FRISBEE allows calculation of product shelf life status at any point of stock rotation, from production to final domestic

storage, offering multiple selection criteria. Shelf life calculation can be performed, based on existing or user defined kinetic data, applying temperature profiles, either retrieved by the Cold Chain Database (CCD, <https://frisbee-wp2.chemeng.ntua.gr/coldchaindb/>) or user-defined. CCD comprises data from industry, cold chain parties (distributors, retailers) and consumer surveys, including all stages of the cold chain (from production to consumption), while the option of Monte Carlo numerical approach allows for the generation of running simulations and distribution scenarios based on real cold chain data. Another important asset of this user-friendly application is the potential to assess product shelf life, based on results of accelerated tests, by providing the measured shelf life at elevated temperatures, a tool of practical usefulness for the food industry. A variety of different food types, spoilage mechanisms or quality deterioration related indicators and relative kinetic models are the main building blocks of this application, that is constantly being enriched with recent data.

## PP75

### Modelling the probability of *Botrytis cinerea* conidia germination in a strawberry acid-based simulated medium

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This study investigated the effects of storage temperature and water activity (aw) on *Botrytis cinerea* germination, the primary cause of post-harvest decay in strawberries. The aim was to simulate real-world storage conditions to better control fungal growth and reduce strawberry waste. *B. cinerea* germination was examined in a strawberry-simulating medium across temperatures from 5-25°C and aw from 0.920-0.998. The medium, based on modified Potato Dextrose Agar, incorporated natural organic acids present in strawberry fruit and its aw was adjusted with glycerol to achieve desired levels. Conidia germination was monitored microscopically for up to 30d. A modified asymmetric model was fitted to *B. cinerea* germination data, estimating the maximum percentage of viable spores (Pmax, %), the time to reach half-maximum germination ( $\tau$ , days) and the germination rate ( $\mu$ , %/h). Cardinal secondary models were then applied to describe the relationships between  $\mu$  and  $\tau$  with aw.

Water activity significantly influenced *B. cinerea* germination at all tested temperatures. Overall, Pmax ranged from 0.45 to 1.0, while  $\tau$  varied from 0.4 to 10.2d. Lower temperatures (5-15°C) increased  $\tau$  values, while temperatures above 15°C had minimal impact on germination at constant aw. The cardinal models tested successfully described the relationships between  $\mu$  and  $\tau$  with aw. Optimal germination rate ( $\mu_{opt}$ ) and time ( $\tau_{opt}$ ) were estimated as  $5.34 \pm 0.51\%/h$  and 10h at 20°C and aw=0.99. Optimal storage temperatures to reduce fruit waste were estimated to be below 10°C for an aw around 0.97 (95th percentile value for strawberry).

This study demonstrates that *B. cinerea* germination in strawberries can be delayed by reducing storage temperatures and controlling fruit aw. The developed models provide valuable insights for optimizing strawberry storage conditions to inhibit fungal growth and minimize post-harvest losses.