



11th International Conference on  
**Predictive Modelling in Food**  
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# 11TH INTERNATIONAL CONFERENCE ON PREDICTIVE MODELLING IN FOOD (ICPMF11):

## BOOK OF ABSTRACTS

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Ursula Gonzales-Barron

and

Vasco A. P. Cadavez

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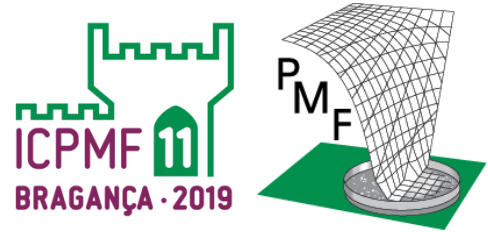
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## PREFACE



Dear Colleagues,

It is our great pleasure to welcome you in Bragança, Portugal, for the **11th International Conference of Predictive Modelling in Food (ICPMF11)**. Since 1992, ten ICPMF editions have taken place, providing a forum for the exchange of ideas, identification of research needs and novel approaches for the advancement of predictive modelling towards ensuring safety and quality of foods.

Bragança is a typically-Portuguese old town (Romanic origin dates back to the 10th century), located by the Natural Park of Montesinho – one of the wildest forest zones of Europe – and the Douro Valley – the third oldest protected wine region in the world; and surrounded by traditional villages of a distinctive rustic beauty. Bragança houses several traditional industries producing a myriad of local foods, such as cheese, fermented meats, wine, chestnuts and honey, which provide substantial economic sustainability to the region.

ICPMF11 reunites food researchers, stakeholders, risk assessors and users of predictive models to present recent developments and trends in modelling approaches for food quality, safety and sustainability. We succeeded to gather a significant number of delegates from over the world to participate in a comprehensive scientific programme that includes keynote lectures, oral communications and posters, allocated in sessions focusing on:

- Advances in predictive microbiology modelling
- Predictive modelling in innovative food processing and preservation technologies
- Advances in microbial dynamics and interactions
- Advances in software and database tools
- Meta-analysis protocols and applications
- Advances in risk assessment methods and integration of omics techniques
- Advances in predictive modelling in food quality and safety
- Predictive mycology
- Individual cell and whole-cell modelling

Apart from those, ICPMF11 features for the first time a special session dedicated to **“Innovative approaches for ensuring safety of traditional foods”** and the Round Table: **“Assuring the Safety of Traditional Foods: A Scientific Contribution to Protecting our Cultural Heritage”**. We, as food researchers based in a Mediterranean mountain region, are aware that the production of traditional foods plays a key role in the development of rural regions, since the agricultural commodities used as raw materials are generally produced locally, allowing and stimulating local commercialisation, thus contributing to a sustainable environment, and employment in rural populations. It was

inspiring for us to have received many submissions from both developed and developing countries on the valorisation of traditional foods through the application of up-to-date modelling research.

Besides that, one morning workshop and three afternoon tutorials were programmed during the day before the scientific programme. The workshop “How to benefit from the Risk Assessment Modelling and Knowledge Integration Platform (RAKIP)” was organised by Matthias Filter. The parallel tutorials “Towards an integrated predictive software map: Practical examples of use of predictive microbiology software tools for food safety and quality”; “Advanced methods in predictive microbiology” and “Topics in quantitative microbial risk assessment using R” were organised by Fernando Pérez-Rodríguez, Pablo Fernández, Alberto Garre and Mariem Ellouze; by Lihan Huang, Cheng-An Hwang and Vasco Cadavez; and by Patrick Njage and Ana Sofia Ribeiro Duarte, respectively. We thank these organisers for their proposals.

Abstracts, reviewed by the ICPMF11 Scientific Committee, are published in the present Book of Abstracts while peer-reviewed original research articles will be invited to be published in ICPMF11 Special Issues in the International Journal of Food Microbiology and Microbial Risk Analysis. To stimulate the participation of postgraduate students and young researchers, two kinds of awards were arranged: the Young Researcher Best Oral Presentation prizes, sponsored by Elsevier; and the Developing Scientist Best Poster prizes, sponsored by the International Committee on Food Microbiology and Hygiene (ICFMH) of the International Union of Microbiological Societies (IUMS). For the first time, this ICPMF edition gives out two awards for the Senior Researcher Best Oral Presentation, sponsored by the open-access journal Foods – MDPI.

In addition to the scientific programme, we prepared an exciting social programme for delegates to appreciate the rich culture, gastronomy and traditions of Bragança, w includes welcome reception, live music, tasting of regional food and a gala dinner in the Castle of Bragança.

We look forward to lively discussions, and hope that this meeting will give you the opportunity to strengthen friendship and cooperation, and build new contacts for future research endeavours.

The ICPMF11 Chairs,

Dr. Ursula Gonzales-Barron

Dr. Vasco A. P. Cadavez





## INTERNATIONAL UNION OF MICROBIOLOGICAL SOCIETIES (IUMS)

COMCOF



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# INTERNATIONAL COMMITTEE ON FOOD MICROBIOLOGY AND HYGIENE (ICFMH)

*Since 1953*

The **International Committee on Food Microbiology and Hygiene (ICFMH)** was founded in 1953 and officially represents International Union of Microbiological Society (IUMS) in all issues related to food microbiology. The ICFMH has observer and/or advisory status in activities of FAO and WHO, ISO working groups related to the detection and enumeration of microorganisms in food.

## ICFMH mission

The major scope of the ICFMH is to contribute to food safety internationally, by means of symposia (e.g., FOOD MICRO), workshops, support of international bodies in food safety issues, publications, and by supporting and initiating education and training in food microbiology. The ICFMH particularly focuses on the food safety situation in developing countries.

## ICFMH main activities

**FOODMICRO Conference.** The initially small ICFMH Symposia and expert meetings developed into the international biennial "FOODMICRO" Conference. The ICFMH and the national organisers managed to keep track with increasing challenges and complexity posed by diverse areas of Food Microbiology.

FOODMICRO 2020 will be held in Athens (Greece), 7<sup>th</sup>-10<sup>th</sup> September 2020 ([www.foodmicro2020.com](http://www.foodmicro2020.com)). Guidelines for organising future FOODMICRO conferences can be found [here](#).

**International Journal of Food Microbiology (IJFM).** As the "flagship" of the ICFMH, and published by Elsevier, it has gained and enjoys constantly increasing international recognition.

**Scholarships.** The ICFMH regularly sponsors qualified countries to participate in international meetings and workshops such as FOOD MICRO. For more information visit: [www.icfmh.org](http://www.icfmh.org).

**ICFMH Mobility Grants** are short-term fellowships annually sponsored to assist young scientists active in food microbiology and hygiene in pursuing research at a host laboratory.

**ICFMH awards** for best student communication (oral and/or poster) are regularly sponsored in international meetings and symposia, including FOOD MICRO.

**Subcommittee on Predictive Modelling in Food (ICPMF).** In 2014, the Executive board agreed to incorporate the **International Committee on Predictive Modelling in Food (ICPMF)** as a subcommittee. The ICPMF has as mission to catalyse the development of predictive modelling in foods, primarily through advancing the success and sustainability of the biannual ICPMF-conferences.

**Working Party on Quality Assurance and Quality Monitoring of Culture Media (WPCM)** was initiated in 1978 to elaborate a "Pharmacopoeia of Culture Media". The 3<sup>rd</sup> edition of the *Handbook of Culture Media for Food and Water Microbiology* was published by The Royal Society of Chemistry (RSC) early 2012.

**Working Party on Advanced Education in Food Microbiology (WPAEFM)** aims to provide a forum for discussion and structuring curricula in food microbiology so that a harmonized set of proposals can be used during discussions with relevant authorities, national bodies and academicians offering courses in food microbiology and food safety.

**Workshops and other activities.** Workshops on special issues in food microbiology and food safety form part of the international scope of ICFMH. Workshops on Food Safety in Africa have been conducted in South Africa (2003 and 2007) and in Ghana (2014) and Cambodia (2018). Workshops on Traditional Fermented Foods were held in Burkina Faso, Denmark and France in 2009, 2010, 2014, 2016 and 2018, respectively. Within the IUMS Outreach Program, a Food Safety Workshop was held in Bali (2011) and Yogyakarta (2017), Indonesia.

## National Delegates and representatives

In order to achieve its goals, it is essential to collaborate with National Societies for Microbiology throughout the world, and to interact with research groups and scientists dealing with food microbiology. We encourage you to directly contact the National Delegate of your country for any issues related to Food Microbiology.

We invite you to visit [www.icfmh.org](http://www.icfmh.org), and to inform and update yourself on the ICFMH. Please do not hesitate to [contact](#) us if you believe valuable information could be uploaded in our website.



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# PLENARY LECTURES

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**Time: 8:30 - 9:10**

**Date: 18th September 2019**

## **64: BAYESIAN MODELLING IN QMRA: SEPARATED BUT TOGETHER, THE GAINS AND THE PAINS**

**Jukka Ranta**

Finnish Food Authority, Helsinki, Finland

**Introduction:** Bayesian models aided with computational tools have been around roughly since 1990's. The expansion to new fields has been gradual with increasing number of applications. While the early applications required programming skills for Monte Carlo sampling methods, specialized tools have substantially eased the burden, allowing focusing more on the modelling itself rather than numerical methods. Nevertheless, they require knowing the underlying principles, ability to build hierarchical models and a careful assessment of the outputs.

**Methodology:** In contrast to classical statistics, Bayesian statistics employs probability distributions to describe all uncertain quantities. This has two important implications: (1) not knowing e.g. a population mean makes us uncertain to an extent that depends on what evidence we have at our disposal. (2) when that evidence changes, so does our uncertainty, expressed in the form of a probability distribution. Hence, a Bayesian model is not only the conditional probability model of data variables, given the parameters, but a full model of both data and parameters from which we can then infer the 'unknowns', given the 'knowns' via Bayes theorem.

**Results:** Foodborne hazard intake variability follows from the variability in food consumption and in pathogen occurrence over multiple sources. These are always subject to uncertainty due to limited data, and often low concentrations. Likewise, a microbiological criterion has an uncertain selective effect on the quality of accepted products due to limited baseline data and sampling properties of the criterion. A hierarchical Bayesian model combines evidence from separate data sources together. Examples are presented.

**Conclusion and Relevance:** In probabilistic QMRA of food production chain, variability of contamination between e.g. batches, units, samples are described by conditional distributions with unknown parameters. This is often implemented as Monte Carlo simulation flowing 'forwards', with no specific method for statistical inference. A Bayesian inference provides multidimensional uncertainty distribution of parameters. Prediction uncertainty and variability can then be assessed separately but together. The vast flexibility of Bayesian methods is both our challenge and our opportunity. Bayesian

modelling is a versatile approach for transparent QMRA and consistent evidence synthesis of available data, but it requires some practice with specialised software.

Keywords: Bayesian inference; evidence; uncertainty; variability; QMRA

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**Time: 14:00 - 14:40**

**Date: 18th September 2019**

## **124: ESTIMATING PARAMETERS FROM DYNAMIC DATA: ADVANTAGES AND CHALLENGES**

**Kirk Dolan**

Michigan State University, East Lansing, USA

Much progress has been made on estimation of microbial growth and decay kinetic parameters under constant conditions. Using two-step modelling, the parameters are estimated usually from linear models, without recursion. These parameters are typically used to predict results under dynamic-temperature environments. One of the greatest successes with two-step modelling has been its application to the food canning industry. Advantages of two-step modelling include being able confirm the trend of the secondary model visually, and the ease of doing linear regression. Disadvantages include the experimental difficulties of attaining constant-temperature conditions at elevated temperatures, and the increased number of experiments required.

Some food processes, such as aseptic and baked waffles, are too fast and too high a temperature to simulate using isothermal experiments. Other processes with slow heating rates can lead to sublethal adaption of microorganisms during dynamic heating. These and other food systems may benefit from one-step regression on the combined primary and secondary models to estimate the microbial inactivation/growth kinetic parameters. Advantages of the one-step method include fewer experiments, easier experimental set-up, no need to consider come-up-time, and ability to use all the data. Disadvantages include not being able to “see” the secondary model, and the increased level of statistical knowledge and software tools required.

A systematic method or “checklist” for estimating parameters will be presented. Key to the method is plotting scaled sensitivity coefficients to determine which parameters can be estimated, which will be correlated, and which will be most accurate. Trouble-shooting tips will be given. Examples of how to estimate parameters will be shown for inactivation (non-isothermal experiments with different heating rates), and for growth (*Listeria* in cheese). A user-friendly method to determine optimal experimental duration will be demonstrated.

The goal of the presentation is to provide a template for other researchers to consider using when they wish to estimate parameters from nonlinear models. The author hopes that the methods presented will mitigate typical roadblocks, such as certain parameters not being estimated, and lack of model convergence.

Keywords: parameter estimation; microbial inactivation/growth; one-step modelling; scaled sensitivity coefficients; non-isothermal

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**Time: 8:40 - 9:20**

**Date: 19th September 2019**

## **42: BIG DATA IN FOOD SAFETY: OPPORTUNITIES AND CONSTRAINTS**

**Hans Marvin, Yamine Bouzembrak**

Wageningen Food Safety Research, Wageningen, Netherlands

Food supply chains are complex and vulnerable to many factors having a direct and/or indirect effect on the development of a food safety risk. To ensure safe foods, representative samples are taken at various stages in the food supply chain, shipped to sophisticated laboratory facilities where the samples are analyzed by highly trained technicians for compliance with the legislation. Challenges in this system are where to control what and when and the high costs. New developments in big data and technology show promising perspectives to solve some of these challenges.

Big data analytics may provide tools to integrate the factors driving food safety risks in a systemic manner, which also will take advantage of the vast amount of data that is available. Recent developments have shown that Bayesian Networks (BNs) allow such approach. BN prediction models have been developed to predict the presence of food safety hazards in various supply chains, to predict food fraud type and to select the sampling site. The models integrated monitoring data with data from influencing factors such as climate, economy or agricultural parameters and often high prediction accuracies are obtained (generally > 90%).

New technology developments in mobile and hand-held devices such as smartphone-based sensors or transportable devices aimed for on-site analysis of a sample and a confirmation decision (hence lab to the sample approach) may improve the efficiency of the food safety control system as well. These on-site detection technologies, together with improved data aggregation, analysis and smart decision support systems, will lead to a paradigm shift in improved food safety control systems and authenticity.

Keywords: Food safety; Bayesian networks; prediction models; on-site analysis

**Time: 14:00 - 14:40**

**Date: 19th September 2019**

### **132: INTEGRATING NEXT GENERATION SEQUENCING INTO MICROBIAL RISK ASSESSMENTS**

**Francis Butler**

UCD School of Biosystems and Food Engineering, University College Dublin, Dublin, Ireland

This talk will highlight the role that next generation sequencing has in undertaking microbial risk assessments. Next generation sequencing has unique possibilities in terms of ‘fingerprinting’ and characterising the microbial pathogens present in foods. The challenge is how to integrate this often-large amount of data into quantitative risk assessments. The presentation will explore how genomic data impacts on the key elements of microbial risk assessment – hazard identification, exposure assessment, hazard characterisation and risk characterisation. The talk will give some case studies of using next generation sequencing to track microbial hazards in food process facilities.

Keywords: Fingerprinting; genomic data

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**Time: 9:00 - 9:40**

**Date: 20th September 2019**

### **119: CHALLENGES AND OPPORTUNITIES IN QUANTITATIVE MICROBIAL RISK ASSESSMENTS FOR VIRUSES**

**Donald Schaffner, Robyn Miranda**

Rutgers University, New Brunswick, USA

Most microbial risk assessments have focused on bacterial pathogens, but there is increased interest in risk assessments for enteric viruses, in part because measures used to control or bacterial contamination in food or water are not always effective for controlling viruses. Viral risk assessments may consider factors such as virus inactivation over time, susceptibility to disinfectants, differences in host immunity, differences in clinical symptoms and health outcomes (including the potential for asymptomatic and secondary infections), genetic diversity and emergence of novel viral strains. Over the last decade, almost two dozen food- or waterborne viral risk assessments have been published, often focusing on commonly contaminated foods and environmental sources associated with outbreaks. Early risk assessments focused on irrigation water quality and a variety of viruses, while more recently published risk assessments have focused on Norovirus in different food products.

Some enteric viruses infecting humans are difficult to culture so surrogate viruses are often used. Surrogate microorganisms are typically selected on the basis of their



morphological similarities and/or similar physiological characteristics to the pathogens of interest. Surrogates for pathogenic foodborne enteric viruses include feline calicivirus, murine norovirus, bacteriophage MS2, Tulane virus, porcine sapovirus and poliovirus. There is no one universal one-to-one relationship between specific foodborne viruses and their surrogates. A surrogate suitable for one stress (i.e. heat) might not be ideal for another stress (i.e. sanitizers), and there are multiple other stresses or situations that need to be studied (frozen survival, pH stress, cross-contamination, etc.).

Viruses associated with foodborne disease tend to be those where humans are the natural hosts, therefore effective risk management strategies need to include preventing exposure of foods to human feces or vomitus. Effective control of water treatment, food processing, cleaning and disinfection of surfaces, personal hygiene and hand washing, and/or sanitation are all required in order to control the spread of viruses along the food chain, and vaccination can also be an important risk management strategy, and vaccines against HAV and rotavirus have already been implemented and there are now several candidate vaccines for NoV, although none have currently made it to market.

**Keywords:** Quantitative microbial risk assessment; viruses; surrogates; modelling; risk management

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## ORAL PRESENTATIONS

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### Oral Session 1: Advances in Predictive Microbiology Modelling

Time: 9:10 - 10:20

Date: 18th September 2019

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#### 10: ONE-STEP DYNAMIC INVERSE ANALYSIS AND PREDICTIVE MODELLING FOR MICROBIAL FOOD SAFETY: THE BAYESIAN WAY

Lihan Huang

USDA Agricultural Research Service Eastern Regional Research Centre, Wyndmoor, USA

**Introduction:** Predictive modelling has emerged as an effective tool for ensuring and enhancing food safety. Mathematical models can be used to estimate microbial shelf-life and conduct risk assessment of foodborne pathogens in foods. One-step dynamic analysis is a new inverse analysis method for determining the kinetic parameters of microbial growth and survival directly from dynamic experiments. This method is especially suitable for evaluating the effect of temperature on microbial growth and survival.

**Methodology:** For one-step dynamic analysis, food samples are exposed to dynamic temperature profiles to observe the effect of temperature on microbial growth and survival, which is described by differential equations as a primary model in combination with a secondary model. The kinetic parameters will be determined by inverse analysis using numerical analysis and optimisation. Once the kinetic parameters are determined, Bayesian analysis can be used to construct their posterior distribution. As a stochastic method, Markov Chain Monte Carlo (MCMC) simulation can be used to predict the growth and survival of microorganisms in foods.

**Results:** One-step dynamic analysis and MCMC simulation has been applied to predictive modelling for assessing dynamic growth of *Clostridium perfringens* in cooked chicken during cooling and *Salmonella* spp. in raw ground beef exposed to complex temperature profiles. The results show that Bayesian analysis using MCMC simulation provides significantly improved accuracy of prediction, with the root mean square error (RMSE) within  $\pm 0.25$  log CFU/g.

**Conclusions and Relevance:** Dynamic Bayesian analysis can be a viable method for inverse analysis and predictive modelling in food safety applications.

Keywords: One-step; Inverse analysis; Bayesian analysis; MCMC; Dynamic prediction

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## 25: DESCRIBING UNCERTAINTY IN PREDICTED THERMAL INACTIVATION OF *SALMONELLA* USING BAYESIAN STATISTICAL MODELLING

Kento Koyama<sup>1</sup>, Zafiro Aspidou<sup>2</sup>, Konstantinos Koutsoumanis<sup>2</sup>, Shige Koseki<sup>3</sup>

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**Introduction:** Uncertainty analysis is the process of identifying limitations in scientific knowledge and evaluating their implications for scientific conclusions. Risk assessors should examine in a systematic way every part of their assessment in order to identify all uncertainties, including those related with the inputs to the assessment and the methods used in the assessment. In the context of microbial risk assessment, the uncertainty in the predicted microbial behaviour can be an important component of the overall uncertainty. Conventional deterministic modelling approaches which provide point estimates of the pathogen's levels cannot quantify the uncertainty around the predictions. The objective of this study was to use Bayesian statistical modelling for describing uncertainty in predicted microbial thermal inactivation of *Salmonella enterica* Typhimurium DT104.

**Methodology:** A set of *Salmonella enterica* Typhimurium DT104 thermal inactivation data in broth with  $a_w$  adjusted to 0.75 at 9 different temperature conditions was obtained from the Combase database ([www.combase.cc](http://www.combase.cc)). Data at 8 temperature conditions were used for model development and one temperature for model validation. A log-linear microbial inactivation was used as a primary model while for secondary modelling, a linear relation between the logarithm of inactivation rate and temperature was assumed. For comparison data were fitted with a two-step and a global Bayesian regression. Posterior distribution of model's parameters were used to predict *Salmonella thermal inactivation* using R, Stan and rstan packages of R software.

**Results:** The model described successfully uncertainty in predicted thermal inactivation. The global regression approach resulted in less uncertain predictions compared to the two-step regression. Combination of the joint posterior distributions allowed to express variables such as cell density with time, total reduction time and inactivation rate as probability distributions at different temperature conditions. The validation of the model also showed that most observed data were within the 95% prediction intervals of the model.

**Conclusion and Relevance:** The model developed using Bayesian regression can describe the uncertainty in predicted thermal inactivation of *Salmonella*. The model provides prediction in the form of probability distributions and can be used to quantify uncertainty related to model fitting in risk assessment studies.

Keywords: Uncertainty; probability; fitting; predictive model; Bayesian regression

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## 55: GUIDELINES FOR (OPTIMAL) EXPERIMENTAL DESIGN OF MICROBIAL INACTIVATION EXPERIMENTS

José L. Peñalver<sup>1</sup>, Alberto Garre<sup>1</sup>, Arantxa Aznar<sup>1</sup>, Alfredo Palop<sup>1</sup>, Pablo S. Fernández<sup>1</sup>, Arturo Esnoz<sup>1</sup>, José A. Egea<sup>2</sup>

<sup>1</sup>Universidad Politécnica de Cartagena, Cartagena, Spain. <sup>2</sup>Centro de Edafología y Biología Aplicada del Segura-CSIC, Murcia, Spain

**Introduction:** Isothermal experiments are nowadays the most popular way to characterize microbial inactivation. They consist in several experiments carried out at different temperatures, whose results are used to estimate the inactivation rate (e.g. D-value) and how it varies with temperature (z-value). Despite requiring simpler equipment than dynamic experiments, they are more complex from the point of view of (optimal) experiment design (OED). This is due to the fact that the design space is bi-dimensional (time & temperature) and the detection limit restricts a maximum duration of the experiment that depends on temperature. Although OED has been applied to dynamic experiments, no methodology is available for a set of isothermal experiments to estimate the D and z-value (or equivalent parameters) using a one-step algorithm.

**Methodology:** A methodology for the experimental design of isothermal inactivation experiments has been developed. It finds the most informative sampling points in a bi-dimensional design space (time & temperature) considering the temperature dependency of the detection limit. It is based on the optimisation of a metric of the Fisher Information Matrix (its determinant; D-criterion). The detection limit is considered via a penalty function in the optimisation problem.

**Results:** OEDs with different number of sampling points have been calculated for three inactivation models (Bigelow, Mafart and Peleg) and three reference microorganisms. Clear patterns have been identified for each model that are independent of the number of sampling points and microorganism analysed. Samples at the highest and lowest temperatures are the most informative ones. Regarding the sampling time, samples close to the detection limit are the most informative ones. The precision of the designs proposed has been compared to equivalent uniform designs, obtaining parameter estimates with lower uncertainty for the same experimental effort.

**Conclusion and Relevance:** The methodology developed generates experimental designs that are more accurate than equivalent uniform designs. Informative design patterns have been identified that are independent of the number of sampling points and the microorganism analysed. The guidelines defined in this research work can be used to design more efficient isothermal inactivation experiments. This would reduce the experimental work required to characterise isothermal inactivation.

Keywords: Optimal experimental design; microbial inactivation; mathematical modelling; foodborne pathogens

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## 51: MODELLING THE EFFECTS OF pH ON GROWTH OF BACTERIA, YEASTS AND MOULDS: TOWARDS A UNIFIED APPROACH

Yvan Le Marc, Nicolas Nguyen Van-Long, Véronique Huchet

Adria Food Technology Institute, Quimper, France

**Introduction:** The “Gamma concept”, based on the observation that factors influencing bacterial growth have multiplicative effects on bacterial growth rate (e.g. temperature, pH, water activity), has been widely used in the field of predictive microbiology. In particular, a number of stochastic models (based on the characterization of individual strains of a single species) have been developed using this concept for Quantitative Microbial Risk Assessment (QMRA) purposes. Most of the predictive models that account for pH effects assume a linear relationship between the bacterial growth rate and suboptimal pH. Among these pH models, one of the most popular is probably the Cardinal pH Model (CPM). However, various studies have challenged this hypothesis and various alternative models have been proposed since. They usually introduce new shape parameters describing a non-linear relationship between pH and maximum growth rate at suboptimal and/or super-optimal pH levels.

**Methodology:** The objective of this work is to study the performance of publically available models (e.g. Rosso et al., 1995; Presser, 2001; Aryani et al., 2015; Akkermans et al., 2016) for the effects of pH on the growth of different bacteria, yeasts and moulds. The data used in this work consist of more than 70 datasets, most of which from the literature, which describe the relationship between pH and maximum growth rate for 16 bacterial species (including *Listeria monocytogenes*, *Salmonella*, *Bacillus cereus*), 18 fungi species (including *Hyphopichia burtonii*, *Aspergillus flavus*, *Penicillium glabrum*, *Mucor spp.*). Criteria used for model selection include quality of fit, parsimony, biological significance of parameters, stability of parameter estimation and ease of incorporation into stochastic models.

**Results:** The results confirm the hypothesis of a non-linear evolution between pH and suboptimal growth rate for most yeast and mould species as well as for several bacterial species. On the basis of parsimony criteria, common shape parameters may be applied for different strains of the same species, which simplifies incorporation into stochastic models taking into account strain variability.

**Conclusion and Relevance:** The study shows that generic versatile pH models may be applied for bacterial, yeast and mould species, leading towards a unified modelling approach.

**Keywords:** Growth; yeasts; moulds; pH

## **8: CARDINAL PARAMETER MODEL CONTAINING A NEW NISIN TERM TO PREDICT GROWTH OF *LISTERIA MONOCYTOGENES* IN PROCESSED CHEESE**

Veronica Martinez-Rios, Mikael Pedersen, Monica Pedrazzi, Elissavet Gkogka, Jørn Smedsgaard, Paw Dalgaard

National Food Institute (DTU Food), Technical University of Denmark, Kgs. Lyngby, Denmark

**Introduction:** Nisin is a preservative with a well-documented use for the control of sporeforming bacteria in processed cheese. However, little information is available with regards to its protective effect against pathogens such as *Listeria monocytogenes*, when introduced in processed cheese by cross-contamination at the consumer phase. The objective was to develop a mathematical model to predict growth of *L. monocytogenes* in processed cheese containing added nisin.

**Methodology:** Minimum inhibitory concentration (MIC) values for nisin were determined experimentally in broth at pH 5.5 and 6.0 and collected from literature at different pH values. A polynomial MIC-function was developed to describe the effect of pH on nisin MIC values. Two existing growth and growth boundary models were expanded with the new MIC-function for nisin to predict growth of *L. monocytogenes* in chemically acidified cheese and processed cheese. To generate growth data for model evaluation, challenge tests (n=45) were performed with *L. monocytogenes* inoculated in chemically acidified cheeses and processed cheeses containing added nisin (0-25 mg/kg). A LC-MS/MS method was developed and validated to quantify nisin A and Z in cheese.

**Results:** The nisin recoveries ranged from 83 to 110 % for nisin A and from 95 to 113 % for nisin Z. The limits of detection and quantification for both nisin A and nisin Z were 0.04 mg/kg and 0.12 mg/kg, respectively. Applicability of the LC-MS/MS method was tested by analysing 13 different cheeses containing nisin. Five cheese samples contained nisin A at concentrations in the range from 0.16 to 0.19 mg/kg. Evaluation of the model by comparison of observed and predicted growth rates resulted in bias and accuracy factor-values of 1.02 and 1.12 for a total of 18 growth responses in processed cheese. Further studies with higher concentrations of nisin will be beneficial to validate the new nisin MIC-function including the effect of pH on nisin MIC values.

**Conclusions and Relevance:** The developed model can be used to support product development, reformulation or risk assessment of processed cheeses containing nisin A.

**Keywords:** Predictive microbiology; LC-MS/MS; product development; risk assessment

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## Oral Session 2: Predictive Modelling in Innovative Food Processing and Preservation Technologies

Time: 10:50 - 12:10

Date: 18th September 2019

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### 29: A SYSTEMATIC MULTISCALE COMPARATIVE STUDY OF THE COMBINED ANTIMICROBIAL EFFECTS OF COLD-ATMOSPHERIC PLASMA (CAP) AND NISIN AGAINST *ESCHERICHIA COLI* PLANKTONIC CELLS AND BIOFILMS

El Kadri Hani<sup>1</sup>, Jorge Gutierrez-Merino<sup>2</sup>, Philip Thomas<sup>3</sup>, Gavin Sandison<sup>4</sup>, Thomas Harle<sup>4</sup>, Thomas Wantock<sup>4</sup>, Andrea Lucca Fabris<sup>3</sup>, Eirini Velliou<sup>1</sup>

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**Introduction:** There is an increasing demand for effective decontamination of food with minimal processing techniques such as the use of natural antimicrobials and Cold Atmospheric Plasma (CAP) to ensure high product quality. Nisin is the only bacteriocin that has been permitted to be applied in food as affirmed by the FDA. However, Gram-negative bacteria such as *Escherichia coli* are resistant to nisin due to their outer membrane covering the cytoplasmic membrane and peptidoglycan layer. CAP is a partly ionized non-thermal plasma generated at atmospheric pressure which creates reactive neutral species that can damage the bacterial membrane. This study aims to investigate the efficacy of a combined CAP/nisin treatment against planktonic cells and biofilms of *E. coli* to improve the antimicrobial activity of nisin.

**Methodology:** *E. coli* (10<sup>9</sup>CFU/mL) grown on biphasic Xanthan gum/Whey protein-based viscoelastic systems<sup>1</sup> that mimic the structure of real food were exposed to CAP (15 seconds) prior to treatment with nisin (50-2,000 IU) for 3 hours. For biofilms, the contaminated biphasic viscoelastic systems were incubated at 37°C to form a layer of biofilm prior to CAP/nisin treatment. Viability was assessed by plate counts and the inactivation kinetics were analysed using GinaFit software while flow cytometric analysis was employed to quantify live/injured cells and reactive oxygen species. Fluorescent microscopy and SEM was used to observe morphological changes and microbial membrane damage.

**Results:** So far, the results show that CAP has antimicrobial effects against planktonic *E. coli* while no effect was observed against biofilms. Fluorescent microscopy images reveal compromised membrane integrity after exposure to CAP shown as an increase in uptake of propidium iodide (membrane integrity indicator). This is further confirmed using

Scanning Electron Microscopy. A combined CAP/nisin treatment resulted in synergistic effects against *E. coli* as compared to each treatment alone.

**Conclusion and Relevance:** This work shows that the antimicrobial activity of nisin can lead to better inactivation of Gram-negatives by pre-treatment with CAP. The results are of significance to the food industry for the effective decontamination of food products using natural antimicrobials.

**References:** (1) Costello, K.M. *et al*, 2018. Int.J.Food.Microbiol. 286,15-30.

**Keywords:** Cold-atmospheric plasma; nisin; *Escherichia coli*; flow cytometry; SEM

### 34: MODELLING THE HEAT INACTIVATION OF *S. NAPOLI* AND *E. HERBORATORIUM* IN LOW MOISTURE FOODS

Joost Smid, Alejandro Amezcuita, Guus Rijke, Christine van de Swaluw, Joerg Ueckert, Erik de Vries, Annemarie Pielaat

Unilever R&D, Vlaardingen, Netherlands

**Introduction:** Thermal inactivation of pathogenic and spoilage organisms in low moisture foods (aw 0.6-0.9) is critically important for guaranteeing microbiological safety and stability. Producers tend to reduce salt because of nutritional health considerations, but it is unclear how this affects microbial inactivation rates during pasteurisation. In this study we evaluate the shape of inactivation curves and the applicability of decimal reduction times (D-values). We predict the time to achieve a 6-log reduction for *Salmonella Napoli* and the *Eurotium herbariorum* mould spores and their relationship with product characteristics.

**Methodology:** We tested 31 design products for heat inactivation of *S. Napoli* and 31 design products for heat inactivation of *E. herbariorum*. We used Bayesian inference to fit a Weibull inactivation model to the data, accounting for biological variability and experimental uncertainty. We determined the relation between the model parameters, pasteurisation temperature and product characteristics (aw, NaCl, sucrose and oil concentrations) to predict the time to 6-log reduction.

**Results:** Inactivation curves were non-linear for many test products: convex for *S. Napoli* and concave for *E. herbariorum*. This indicates the existence of more heat-resistant subpopulations of *S. Napoli* and an increased susceptibility to extended heating for *E. herbariorum*. Lower pH-values resulted in 0.5-1 log shorter times to 6-log reduction for *S. Napoli* at typical pasteurisation temperatures of 75-85°C. For *E. herbariorum*, higher aw and percentage sucrose resulted in 1-2 log shorter times to 6-log reduction. By the Weibull model, computed times to 6-log reductions were 1-2 logs shorter than by the linear model for *S. Napoli*, but not for *E. herbariorum*.

**Conclusion and Relevance:** We parameterised an inactivation model for *Salmonella* and moulds using design products with a broad range of characteristics and showed how using D-values does not always accurately describe the non-linearity of thermal inactivation for



both types of organism. Results of our model can be used to predict heat inactivation as input for the pasteurisation process in factories where low moisture foods are manufactured. Our results can be used by food process managers to give guidance for a proper heat treatment regime of low moisture food products, guaranteeing microbial safety and stability during pasteurisation.

**Keywords:** Modelling; inactivation; *Salmonella*; moulds; predictive microbiology; heat treatment

### **36: ANTIMICROBIAL PHOTODYNAMIC TREATMENT OF ORANGE (*Citrus sinensis* L. Osbeck) PEEL: *ALICYCLOBACILLUS* spp. INACTIVATION AND EFFECTS ON COLORIMETRIC CHARACTERISTICS**

Leonardo do Prado-Silva<sup>1</sup>, Ana T. P. C. Gomes<sup>2</sup>, Mariana Q. Mesquita<sup>2</sup>, Maria G.P.M.S. Neves<sup>2</sup>, Maria A. F. Faustino<sup>2</sup>, Adelaide Almeida<sup>2</sup>, Gilberto U.L. Braga<sup>3</sup>, Anderson S. Sant'Ana<sup>1</sup>

<sup>1</sup>University of Campinas, Campinas, Brazil. <sup>2</sup>University of Aveiro, Aveiro, Portugal. <sup>3</sup>University of São Paulo, Ribeirão Preto, Brazil

**Introduction:** The main source of microbial contaminants that can be found in fruit juices, such as *Alicyclobacillus*, is the soil. Through direct contact or dust, spores of *Alicyclobacillus* can contaminate the peel of fruits used for juice making. As *Alicyclobacillus* spores are highly heat resistant to heat treatment conditions employed for juice processing, fruit juice industry employs alternatives to reduce the contamination in fruit peels prior to juice extraction. Antimicrobial Photodynamic treatment (aPDT) may be an alternative method to chemical methods for *Alicyclobacillus* inactivation on fruit peels.

**Methodology:** Spore suspensions of *Alicyclobacillus acidoterrestris* (DSM 2498) were prepared. The photosensitizer used in this study was a cationic porphyrin (Tetra-Py<sup>+</sup>-Me) at concentration of 20 or 50 µM. The spores (6-7 log spores/mL) were inoculated on the orange peel surface. Then, fruit peels were exposed during 6 h to white LED spotlight or to sunlight, both at an irradiance average of 65 mW/cm<sup>2</sup>. The spore survivors were counted on YSG agar (pH 3.7) after heat-shock (80°C/10 min). Inactivation curves were plotted and compared using ANOVA. The values for L\*, a\* and b\* from each orange peel slice were measured in triplicate using a colorimeter to detect the color change after aPDT treatment.

**Results:** The *ex vivo* aPDT treatment led to 0.7 log reductions (ANOVA, P<0.05) of *A. acidoterrestris* (DSM 2498) after 6 h of treatment using the white LED spotlight. In addition, 2.6 log reductions (ANOVA, P<0.05) were also observed for the DSM 2498 strain when exposed to sunlight. The color parameters (L\*, a\* and b\*) of orange peel changed from 67.08 ± 1.05, 11.32 ± 0.52 and 64.05 ± 1.20 to 70.54 ± 1.52, 12.50 ± 0.56 and 65.34 ± 1.58 after 3 h of exposure to white LED spotlight. In addition, by the exposure to sunlight, color changed from 59.69 ± 0.14, -4.53 ± 0.30 and 52.39 ± 0.62 to 61.63 ± 0.04, 12.81 ± 0.69 and 36.88 ± 1.46.

**Conclusion and Relevance:** This work suggests that aPDT can be a potential method for reduction of *Alicyclobacillus* counts on orange peel. However, further developments and optimisation are required to ensure conditions feasible to implement by the fruit juice industry.

Keywords: PDT; spores inactivation; preservation technology; innovative processing

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## **66: DEVELOPMENT OF A FRESH INDEX AS PREDICTIVE SHELF LIFE INDICATOR FOR MA-PACKED PORK IN GERMAN SUPPLY CHAINS**

Martin Hebel<sup>1</sup>, Antonia Albrecht<sup>1</sup>, Stephanie Krieger-Guess<sup>1</sup>, Baier Achim<sup>2</sup>, Matthias Brunner<sup>3</sup>, Judith Kreyenschmidt<sup>1</sup>

<sup>1</sup>Institute of Animal Science, University of Bonn, Bonn, Germany. <sup>2</sup>arconsis IT Solutions GmbH, Karlsruhe, Germany. <sup>3</sup>tsenso GmbH, Stuttgart, Germany

**Introduction:** Appropriate shelf life assessment and monitoring the actual spoilage status of fresh meat in food supply chains is still challenging. The aim of the Freshindex project is the development and implementation of a dynamic quality Freshindex for fresh pork based on predictive modelling. In combination with temperature monitoring data, the Freshindex displays real-time information about the shelf life of the product.

**Methodology:** The spoilage of more than 350 samples of MA-packed pork loin and steaks was characterized with focus on initial contamination, microbial and sensory spoilage, pH, texture and color. Storage tests under constant and dynamic conditions were conducted to characterize the spoilage kinetics of the food. Based on sensory and microbial models, a Freshindex was established as real-time shelf life indicator. Finally, the implementation of the Freshindex was investigated in pilot trials in a German pork supply chain.

**Results:** As main predictors for the freshness, initial contamination, total viable count (TVC) during storage and sensory attributes were identified. Based on the growth of the TVC, a shelf life model was developed by combining the modified Gompertz and the Arrhenius model. It was matched to a sensory model to scale the information on a Freshindex. Combining sensory and microbiological models was challenging due to the variability of the data. The definition of a combined acceptance level enabled the calculation of the Freshindex. A data cloud was established to provide the required data architecture and enable an online data exchange to calculate the remaining shelf life and the Freshindex, respectively. A mobile APP was developed to provide the actual product information in every step of the chain. In order to model the up scaling of the data, a Virtual Supply Chain was developed covering the Freshindex, logistic processes, flow of goods and sensor data.

**Conclusion and Relevance:** The Freshindex, in combination with adequate temperature monitoring solutions, is considered as an effective tool to reflect the actual status of the product. An upscaling for different supply chains and for further perishable food products is planned as future prospect. This contributes to an improved cold-chain management and long-term prevention of food waste.

Keywords: Predictive microbiology; cold-chain management; shelf-life model; meat quality; FreshIndex

## 80: DESIGN OF CARVACROL-BASED ACTIVE PACKAGING FOR EXTENDING FRESH FISH SHELF-LIFE

Carlos Vilas<sup>1</sup>, Miguel Mauricio-Iglesias<sup>2</sup>, Míriam R. García<sup>1</sup>

<sup>1</sup>Process Engineering group. IIM-CSIC, Vigo, Spain. <sup>2</sup>Department of Chemical Engineering, Univ. de Santiago de Compostela, Santiago de Compostela, Spain

**Introduction:** Active packaging (AP) systems may be used to extend shelf-life by releasing active substances (AS) which inhibit the growth of bacteria responsible for safety and quality changes. The release rate of the active substance depends on the AP material. An adequate selection of AP composition and active substance initial concentration will improve shelf-life.

**Methodology:** We combine mathematical models with optimisation methods to optimally design the AP. The case study used for demonstration is the smart AP of hake using carvacrol as AS. Three materials are considered for the AP: polypropylene (PP); low-density polyethylene (LDPE); and high-density polyethylene (HDPE). The model combines: (i) a partial differential equation model (Fick second law with variable diffusivity) to describe the release of the AS into the food product [1]; and (ii) a system of ordinary differential equations to describe safety/quality changes as a function of storage conditions and AS concentration in the food [2]. *Listeria monocytogenes* concentration, which affects widely consumed products, is used as the safety indicator. KI-value (standard index for early quality degradation) is selected as the quality indicator.

**Results:** The optimal AP design consisted of the following three-layer configuration: LDPE-PP-LDPE. First and last layers are, respectively, in contact with the environment and the food. The optimal size of the layer in contact with the food is the minimum allowed (m) and initial carvacrol concentration on such layer is around 50 kg/m<sup>3</sup>, i.e. half of the maximum allowed. With this configuration, shelf-life may be extended around 24%. Similar results are obtained with two-layer configuration: PP-LDPE.

**Conclusion and Relevance:** We showed that using mathematical models for optimal AP design allows for increasing food shelf-life. Models can be also used for shelf-life prediction so that sellers may adjust prices and reduce food losses. Although tested for hake, the same methodology can be extended to other fish and meat products.

### References:

- [1] Mauricio-Iglesias,M., Guillard,V., Gontard,N., Peyron,S.(2009). *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, 26:1515–1523.
- [2] Vilas,C., Alonso,A.A., Herrera,J.R., Bernárdez,M., García,M.R.(2018). *J Food Eng*, 222: 11-19.

Keywords: Shelf-life; active packaging; optimisation; predictive models

## 90: MODELLING THE BIOPROTECTION OF *LACTOBACILLUS SAKEI* CTC494 AGAINST *LISTERIA MONOCYTOGENES* IN COOKED HAM DURING REFRIGERATED STORAGE

Cristina Serra-Castelló<sup>1</sup>, Jean Costa<sup>2</sup>, Anna Jofré<sup>1</sup>, Araceli Bolívar<sup>2</sup>, Margarita Garriga<sup>1</sup>, Fernando Pérez-Rodríguez<sup>2</sup>, Sara Bover-Cid<sup>1</sup>

<sup>1</sup>IRTA, Food Safety Programme, Monells, Spain. <sup>2</sup>Department of Food Science and Technology, Faculty of Veterinary, Agrifood Campus of International Excellence (CeIA3), University of Cordoba, Córdoba, Spain

**Introduction:** The development and implementation of predictive models to quantify the bioprotective effect of bacteriocin-producing lactic acid bacteria cultures are scarce. The objective of this study was to evaluate and model the interaction between the antilisteria strain *Lactobacillus sakei* CTC494 and *Listeria monocytogenes* CTC1034 in vacuum-packed sliced cooked ham.

**Methodology:** Cooked ham was sliced under aseptic conditions and inoculated with *L. monocytogenes* CTC1034 and/or the sakacin K producer *L. sakei* CTC494 in mono-culture ( $N_0=10^1$  cfu/g) and co-culture at 1:1, 1:3 and 1:5 log ratios of *L. monocytogenes*:*L. sakei*. Samples were vacuum packaged and stored at isothermal (2, 5, 10 and 15°C) and dynamic temperature conditions. The Baranyi and Roberts model was used to estimate the growth kinetic parameters ( $l$ ,  $m$ ,  $N_{max}$ ). The effect of storage temperature was modelled using the hyperbola (for  $l$ ) and Ratkowsky (for  $m$ ) models. The Jameson and Lotka Volterra models were used to characterise and model the interaction between the bioprotective strain and the pathogen. The predictive performance of the interaction models was assessed through the Acceptable Prediction Zone (APZ) approach.

**Results:** In mono-culture, *L. monocytogenes* grew at all temperatures, however *L. sakei* CTC494 inhibited its growth at ratios 1:3 and 1:5 at 2 °C. At higher temperatures, *L. monocytogenes* was able to grow though to a lesser extent when the bioprotective strain was present.  $N_{max}$  of the pathogen was strongly affected with increasing levels of *L. sakei* CTC494. At abusive temperatures (15 °C), *L. monocytogenes*  $N_{max}$  decreased 4 logs, in presence of the bio-protector, as compared to that obtained in mono-culture. The inhibitory effect of the *L. sakei* CTC494 against *L. monocytogenes* was properly characterised using the Lotka Volterra model. The developed interaction model was tested under dynamic conditions, resulting in APZ values  $\geq 85\%$  for ratios 1:3 and 1:5.

**Conclusion and Relevance:** *L. sakei* CTC494 is an effective bioprotectant against *L. monocytogenes* in vacuum-packed cooked ham and the developed interaction model was able to satisfactorily predict the inhibitory effects. Developed models could be used by the industry as predictive tools to assess and establish pathogen control measures in cooked ham.

**Acknowledgements:** SEGURPREDICT (INIA RTA2012-00030)

Keywords: Biopreservation; meat products; food safety; interaction models; bacteriocin

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## Oral Session 3: Advances in Microbial Dynamics and Interactions

Time: 14:40 - 16:10

Date: 18th September 2019

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### 28: QUANTIFYING THE SYNERGISTIC EFFECTS OF NOVEL MICROBIAL INACTIVATION TECHNOLOGIES AND MICROSTRUCTURE ON THE STRESS ADAPTATION AND ANTIMICROBIAL RESISTANCE (AMR) OF *LISTERIA* IN MULTI-PHASE FOOD MODELS

Katherine Costello<sup>1</sup>, Jorge Gutierrez-Merino<sup>2</sup>, Madeleine Bussemaker<sup>1</sup>, Cindy Smet<sup>3</sup>, Jan Van Impe<sup>3</sup>, Eirini Velliou<sup>4</sup>

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**Introduction:** Minimal food processing methods, e.g. cold atmospheric plasma (CAP), ultrasound and/or use of natural antimicrobials, are of interest to replace traditional decontamination techniques. However, the mechanisms of action of combined treatments remain unclear. Solid(like) foods vary significantly in chemical composition and rheological/structural properties, which can impact non-thermal processing efficacy, and diffusion/efficacy of natural antimicrobials. These properties can impact environmental stress adaptation and cross-protection and may lead to different levels of AMR. Furthermore, *Listeria* is often associated with outbreaks in ready to eat foods. Our previous work showed significant structural effects on a microscopic scale, i.e., system viscosity & growth type (surface/immersed) affected *Listeria* colony size and growth location/distribution. *Furthermore, selective surface growth on the protein phase of a complex biphasic protein-polysaccharide model system was observed for the first time*<sup>(1)</sup>. Most studies on the inactivation of pathogens by natural antimicrobials and/or ultrasounds/CAP are conducted in liquids, or in/on food products. However, many foods are solid(like) and studies in real foods are informative only for the product studied. A fundamental study on microbial inactivation by natural antimicrobials combined with ultrasound/CAP in structured food model systems is lacking: our work aims to address this gap.

**Methodology:** *Listeria* was grown as colonies in/on structured gels of varied viscosity (Xanthan gum), with/without added nisin, at 10°C, 30°C and 37°C. The systems were

treated with CAP (dielectric barrier discharge) or ultrasound (44kHz-1MHz), and microbial inactivation kinetics were obtained. Colony size, spatial organisation and treatment effects on a colony/sub-colony level were quantified using advanced microscopy.

**Results:** Effects of system microstructure on the inactivation kinetics are observed for all systems, with differences in cellular/colony morphology observed following treatment. Generally, greater effects are observed in systems containing nisin.

**Conclusion and Relevance:** This work sheds light on the combined efficacy of novel processing techniques for the inactivation of *Listeria* in/on structured food models, highlighting the importance of accounting for structural effects when designing inactivation processes for the food industry.

**References:** (1) Costello, K.M. *et al*, 2018. *Int.J.Food.Microbiol.* 286,15-30.

Keywords: Nisin; plasma; ultrasound, multi-scale modelling

## **58: SPOILAGE INDICATORS IN FRESH PORK OR POULTRY SAUSAGES: EFFECTS OF MODIFIED ATMOSPHERE PACKAGING AND POTASSIUM LACTATE.**

Ngoc-Du Martin Luong<sup>1</sup>, Sabine Jeuge<sup>2</sup>, Louis Coroller<sup>3</sup>, Carole Feurer<sup>4</sup>, Marie-Hélène Desmonts<sup>5</sup>, Nicolas Moriceau<sup>1</sup>, Valérie Anthoine<sup>1</sup>, Sophie Gavignet<sup>5</sup>, Adeline Rapin<sup>5</sup>, Emeline Robieu<sup>2</sup>, Monique Zagorec<sup>1</sup>, Jeanne-Marie Membré<sup>1</sup>, Sandrine Guillou<sup>1</sup>, Consortium Redlosses<sup>6</sup>

<sup>1</sup>SECALIM, INRA, ONIRIS, Université Bretagne Loire, Nantes, France. <sup>2</sup>IFIP, French Institute for the Pig and Pork Industry, Maison-Alfort, France. <sup>3</sup>Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (LUBEM), UMT Alter'ix, Quimper, France. <sup>4</sup>IFIP, French Institute for the Pig and Pork Industry, Le Rheu, France. <sup>5</sup>Aerial, Parc d'Innovation, Illkirch, France. <sup>6</sup>SECALIM, IFIP, LUBEM, MICALIS, ITAVI, MAIAGE, U.LIEGE, Nantes, France

**Introduction:** The spoilage of meat products is mostly caused by the microbial contamination. Packaging under modified atmosphere (MAP) or addition of preservatives are commonly used by manufacturers to delay spoilage. This study aimed to describe effects of potassium lactate and MAP on several spoilage indicators in fresh pork or poultry sausages.

**Methodology:** Experimental data were obtained from ten independent batches of fresh poultry and pork sausages. For each batch, sausages were made with three lactate formulations and packed under three MAP (Air, MAP1:30%CO<sub>2</sub>-70%O<sub>2</sub>, MAP2:50%CO<sub>2</sub>-50%N<sub>2</sub>). Different spoilage indicators were monitored during chilled storage until the 22<sup>nd</sup> day after production: pH, total aerobic mesophilic bacteria (TAMB), lactic acid bacteria (LAB), off-odour evaluation by trained panellists, visual defects rating and chromametric measurement. Effects of MAP and lactate on these indicators were assessed by fitting linear mixed effects models on experimental data, taking into account additional random effects for batches of production.

**Results:** Effects of lactate and MAP on each spoilage indicator depended on meat matrices. Acidification, discolouration or the increase of off-odours intensities and TAMB were observed earlier in poultry than in pork sausages. In poultry sausages, MAP 50%CO<sub>2</sub>-50%N<sub>2</sub> decreased the off-odour perception but intensified acidification. In contrast, in pork sausages, acidification was only influenced by lactate addition, and the off-odour perception was only influenced by MAP. Packaging swelling depended strongly on MAP: no swelling was observed for poultry sausages under 30%CO<sub>2</sub>-70%O<sub>2</sub>. Finally, evolution of LAB was affected only by MAP in poultry sausages, and only by lactate in pork sausages.

**Conclusion and Relevance:** Spoilage was observed earlier in poultry sausages than in pork. Nevertheless, the monitored indicators did not suggest common general relationships for poultry and pork sausages, between manufacturing techniques (MAP, lactate) and spoilage occurrences. Further studies on spoilage should investigate on integrative data such as bacterial metabolism and ecosystem structure. Mixed effects models enabled to take into account non-independencies between conditions from the same batch and random variabilities between batches.

**Keywords:** Meat spoilage; modified atmosphere packaging; potassium lactate; linear mixed effects models; quantitative analysis

## **88: EVALUATION OF NEGATIVE BINOMIAL MODEL TO DESCRIBE THE INACTIVATION KINETICS OF VEGETATIVE PATHOGENS IN THERMALLY PROCESSED LOW MOISTURE FOODS**

Balasubrahmanyam Kottapalli, Tim Perez

Conagra Brands, Omaha, USA

**Introduction:** Log-linear regression models are widely used to determine the heat resistance (ex: D- and z-values) of pathogens. These models assume first-order kinetics however, factors such as strain variability, starting inoculum, matrix, microbe-microbe interactions and other environmental factors may cause the pathogens to display non-linear kinetics. Hence, it is very critical to accurately estimate the heat resistance of pathogens given food industry's obligation for a validated thermal process to comply with regulations. Negative binomial models have been previously proven to be superior to linear and non-linear models which are commonly used to describe bacterial count data. The purpose of this study was to evaluate negative binomial model to describe the inactivation kinetics of *Salmonella* spp. and *Listeria monocytogenes* in 3 low moisture food matrices.

**Methodology:** Peanut butter-filled (PB) pretzels, whole wheat (WW) pita chips and sunflower seeds samples were individually inoculated with multi-strain cocktails of *Salmonella* spp. or *L. monocytogenes*. PB pretzels and WW pita chips were baked at 148.9°C and 176.6°C for 0-30 min. Sunflower seeds were roasted at 107.2°C and 135.0°C for 0-45 min. Following treatments, samples were enumerated for pathogens using scientifically valid methods. A negative binomial non-linear exponential decay model was fit separately for *Salmonella* spp. and *L. monocytogenes*, using PROC NLMIXED in SAS

software. The fit of this exponential decay model was compared to linear and several other non-linear models using likelihood ratio tests. This model was deemed to be a significantly better fit at an  $\alpha=0.05$ .

**Results:** Data analysis indicated that negative binomial model described inactivation kinetics in both pathogens better than other linear and non-linear models. Negative binomial model was also used to estimate baking/roasting times to achieve a 4-log and 5-log reduction in both pathogens along with 95% inverse prediction intervals.

**Conclusion and Relevance:** The study findings indicate that food processors when establishing process safety, must rely on alternative approaches when log-linear models cannot accurately estimate microbial destruction during process validation studies. The approaches we used in this study will have substantial practical importance to food manufacturers of thermally processed foods, allowing them to vary their thermal treatment of ready-to-eat products in a safe manner.

**Keywords:** *Listeria monocytogenes*; log-linear model; *Salmonella* spp.; negative binomial model

## 96: IMPROVED PROBABILISTIC SIMULATIONS OF *BACILLUS CEREUS* GROUP III

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**Introduction:** When using the cardinal model, several studies proved the existence of correlations between the strain dependent parameters of the cardinal temperature model ( $T_{\min}$   $T_{\text{opt}}$   $T_{\max}$ ) and the broth parameter  $\mu_{\text{opt\_Broth}}$  but only few take them into account in simulations. Our objective is to fill this gap.

**Methodology:** Cardinal temperature values (denoted as above) and the optimum specific growth rate  $\mu_{\text{opt\_Broth}}$  were obtained for 15 *B. cereus* Group III strains. A linear correlation was derived for  $\mu_{\text{opt\_Broth}}$  as a function of the cardinal temperatures, and the relationship was validated with external literature data. In parallel, challenge tests were performed on three batches of a vegetable puree mix inoculated with the reference strain F4810/72 and stored at different temperatures ranging from 12 to 46°C. Maximum specific growth rates were derived and used to assess their ratio (called Food Factor,  $F_f$ ) to  $\mu_{\text{opt\_Broth}}$ . Monte-Carlo simulations were performed to sample from the different distributions of the cardinal parameters, then the  $\mu_{\text{opt\_Broth}}$  were calculated according to the above relationship. Finally, the simulation results were compared to new experimental data obtained via a different strain and batch. The effect of the parameter distributions on the overall performance of the model was investigated by sensitivity analysis.

**Results:** Strong correlations were observed between  $\mu_{\text{opt\_Broth}}$  and  $T_{\text{opt}}$  (0.91) and  $T_{\min}$  (-0.87). The distributions of the parameters ( $T_{\min} \sim N(8.2, 0.9)$ ,  $T_{\text{opt}} \sim N(40.7, 1.9)$ ,  $T_{\max} \sim N(48.9, 1.2)$ ) and the average  $F_f=0.61$  allowed to generate simulated



maximum specific growth rates for different temperature levels. The validation data set fell within the upper band of the simulated growth rates, which showed increased variability as we moved closer to the optimal temperatures. The sensitivity analysis showed that, apart from the temperature, the factors  $\mu_{\text{opt\_Broth}}$ ,  $T_{\text{min}}$ ,  $T_{\text{opt}}$  and  $T_{\text{max}}$  had similar effects on the simulated growth rates, however, the standard deviation of  $T_{\text{min}}$  had a greater impact on the results variability.

**Conclusions and Relevance:** The newly developed model proposes a generic approach to better predict the growth of *B. cereus* Group III strains. Future validations will run with more strains and extend the concept to other matrices to provide robust exposure assessments supporting informed food safety decisions.

Keywords: Probabilistic; *B. cereus*; cardinal; temperature; correlation

## 97: INTEREST AND LIMITATION OF USING THE WEIBULL MODEL TO DESCRIBE AND QUANTIFY THE INACTIVATION KINETICS OF MICROORGANISMS

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**Introduction:** The Weibull model or cumulative function of the Weibull distribution is a model increasingly used to describe and quantify the no log linear inactivation kinetics of microorganisms. The Weibull model is a simple two-parameter model, with slope and shape parameters. The use of the Weibull model requires certain precautions and its misuse may affect the analysis of the data. This presentation provides a guide to using the Weibull model through various examples.

**Methodology:** Heat treatment inactivation kinetics, from different data set, with different shapes will be fitted with the Weibull model using a single slope parameter or not. Secondary models will be adjusted on the adjusted parameters of the Weibull model according to the values of the environmental factors temperature and pH.

**Results:** The fit quality of the primary and secondary models was evaluated on different data sets given from our results or from the bibliography. It generally appears that environmental conditions do not affect the shape parameter. On the other hand, the use of a single value of the shape parameter improves the fit of secondary models.

**Conclusion and Relevance:** This approach limits the structural correlation between the two Weibull model parameters and improves the fit of secondary models describing the influence of environmental factors on the slope parameter. This use of the Weibull model could be recommended to describe, to fit and to quantify any type of microbial inactivation kinetics, thermal or non-thermal.

Keywords: Inactivation; Weibull model; Secondary modelling

### 103: A MATHEMATICAL APPROACH FOR INVESTIGATING THE EFFECT OF FOOD MATRIX ON THE BIO-PROTECTIVE CAPACITY OF LACTOBACILLUS SAKEI CTC494 AGAINST *LISTERIA MONOCYTOGENES* IN READY-TO-EAT FISH PRODUCTS

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**Introduction:** the use of antagonistic microorganisms, i.e. lactic acid bacteria, against *Listeria monocytogenes* is an effective, and chemical free, bio-preservation strategy to control the pathogen growth in ready-to-eat products. The study aimed to investigate the effect of food matrix on the bio-protective capacity of *Lactobacillus sakei* CTC494 on *Listeria monocytogenes* based on a predictive microbiology approach.

**Methodology:** Experiments were carried out in mono and co-culture using a concentration of 2 log cfu/g for *L. monocytogenes* CTC1034 and 2 and 4 log cfu/g for *L. sakei* CTC494, respectively, generating the ratio 1:2 in co-culture. Both products were stored at 2 and 12 °C. Baranyi and Roberts model was used to obtain the growth parameters of each microorganism in mono-culture. The interaction between *L. sakei* CTC494 and *L. monocytogenes* CTC1034 was predicted from co-culture data using the Jameson effect, Jameson effect modified and Lotka-Volterra models.

**Results:** The comparison of the kinetics in mono- and co-culture allowed to quantify the pathogen inhibition in all conditions studied. In surimi, the bio-protection effect was only evident on the maximum population density ( $N_{max}$ ), with a drop from 8.4 to 6.7 log cfu/g. By contrast, lag time ( $l$ ) was longer in mono-cultures, particularly at 2 °C. In pâté, outcomes demonstrated a greater inhibition of the pathogen, with all kinetic parameters being affected, especially the  $N_{max}$ , which decreased from 7.3 to 4.1 log cfu/g. Results in surimi implied an antagonism–mutualism continuum between both microorganism, which was not observed in pâté. The microbial interaction observed in both products stored at 2 °C was properly described by Lotka-Volterra model (RMSE<0.6). At 2 °C, the values obtained for the interaction parameter ( $f$ ) confirmed the synergistic (i.e. <0) and antagonistic (i.e. >1) effects observed in surimi and pâté, respectively.

**Conclusion and Relevance:** Results suggest that food matrix could play an important role in the effectiveness of bio-protective cultures, hence its effect should be considered in the design of bio-preservation strategies. The use of the Lotka-Volterra model could provide a wide range of application thanks to its capacity to account for the antagonism-mutualism continuum.

**Acknowledgements:** AGR20141-906, CNPqGDE229638/2013-9 and TUBITAK-2219.

**Keywords:** Food matrix; RTE fish; bio-protective culture; microbial interaction; antagonism-mutualism effect

## 111: MECHANISTIC MODELLING OF YEAST COMPETITION IN MIXED CULTURE FERMENTATIONS OF WINE

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**Introduction:** Mixed culture wine fermentations combining species within the *Saccharomyces* genus have the potential to produce new market tailored wines and may contribute to alleviating the effects of climate change in winemaking. Species, such as *S. kudriavzevii*, show good fermentative properties at low temperatures and produce wines with lower alcohol content, higher glycerol amounts, and good aroma. However, the design of mixed culture fermentations combining *S. cerevisiae* (strain T73) and *S. kudriavzevii* ( strain CR85) species requires investigating the competitions mechanisms. With this aim, we derived the first mechanistic model recovering individual and mixed culture yeast dynamics at 25°C.

**Methodology:** The experimental set-up consists of biological triplicates from different single and mixed fermentations with different inoculum size. Measurements include relative cellular concentrations by qPCR and extracellular metabolites such as glucose, ethanol, and glycerol. Candidate models were fitted to the data using an adequate simulator and a global optimiser.

**Results:** The model features the following mechanisms: i) cells compete for the two major substrates nitrogen and glucose, where the first is limiting; ii) *S. cerevisiae* appears to enhance substrate transport in the presence of *S. kudriavzevii*, the effect depending on the relative initial amounts; iii) *S. cerevisiae* inhibits substrate transport in *S. kudriavzevii*; iv) *S. cerevisiae* kills *S. kudriavzevii*; v) *S. cerevisiae* is partially capable of reutilizing *S. kudriavzevii* nitrogen. The model parameters differ between species regarding growth and competition for nutrients. The nonlinear expression detailing *S. kudriavzevii* death caused by *S. cerevisiae* is critical in successfully explaining the data. Further, *S. cerevisiae* restart of growth by the onset of *S. kudriavzevii* decline points to the ability of *S. cerevisiae* to recover some nitrogen from its competitor.

**Conclusion and Relevance:** The model provides novel insights into mixed fermentations by *S. cerevisiae* strain T73 and *S. kudriavzevii* strain CR85. Strain T73 always swifts CR85 at 25 °C and appears to have developed various mechanisms that make it highly competitive in the nitrogen-limited wine fermentation environment. A better quantitative understanding of these mechanisms can be used to design new effective mixed processes. The model suggests inoculating cells in sequence to guarantee coexistence.

**Keywords:** Mixed fermentation; mechanistic model; dynamic; non-conventional yeasts

## Oral Session 4: Innovative Approaches for Ensuring Safety of Traditional Foods

Time: 16:40 - 17:40

Date: 18th September 2019

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### 107: THE USE OF RISK-BASED MODELLING TOOLS FOR THE MANAGEMENT OF FOOD SAFETY IN THE FRENCH DAIRY SECTOR: A FEEDBACK

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**Introduction:** Next to GHP, HACCP plan and traceability, Quantitative Microbiological Risk Assessment (QMRA) coupled to predictive microbiology (PM) have emerged as useful tools to manage the safety of food products. Since 2003, the French dairy sector has developed risk-based tools to help dairy manufacturers in this purpose. The objective of this presentation is to provide a feedback on the use of these tools in France.

**Methodology:** Stochastic QMRA models adapted to several cheese technologies and pathogenic bacteria were developed. Inputs are : the milk contamination level (issued from routine data statistical analysis), the physico-chemical parameters during process, challenge-tests results and cardinal values of the bacteria considered. Monte-Carlo simulations allow to consider variability and uncertainty. The outputs of the QMRA models are the contamination level in the products (prevalence, concentration, variability) at each step of the process, the risk of illness, and the probability of detecting non-conformities. A web-based interface ([www.maisondulait.agr.fr](http://www.maisondulait.agr.fr)) was developed to enable dairy operators to perform simulations with their own data.

**Results:** The tools developed were applied in several contexts: to optimise sampling plans, to assess shelf life, to better understand the impact of raw milk contamination and process parameters, to validate food safety management options such as milk sorting, etc. Studies could be performed collectively (several operators together), or individually. Results are confronted to the risk of illness decrease.

**Conclusion and Relevance:** The approach developed by the French Dairy Sector has now reached a mature stage, with French dairy companies being aware of the need of QMRA and PM to perform a risk-based approach. However, increasing the formation of future quality manager and inspectors from the food safety authorities is necessary to make them routine tools. Time and human resources are generally lacking for SME, next to the need of specific and rare competences in mathematical modelling. The need for outputs focused on sampling strategy was clearly observed. Finally, raising awareness on the risk-based

approach and on the tools with concrete applications through a regular communication is of particularly great importance.

Keywords: Food safety; dairy; quantitative risk assessment; predictive microbiology; sampling

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## **69: DETERIORATION AND SHELF LIFE TESTING OF “FOLERE DRINK”, AN ARTISANAL SOFT DRINK FROM NORTHERN CAMEROON**

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**Introduction:** The international market for traditional African food and beverages is growing in Europe and American countries. The success of the use of these foods and beverage is attributed to several factors including globalization, the expansion of the number of immigrants from Southern countries to the Northern one, and the development of African restaurants in large western agglomerations. As a result, the international market for these products is becoming more and more attractive. One of these emerging product is a so called “folere drink” an artisanal soft drink from northern Cameroon. Unfortunately the quality of these products are not yet guarantee. This work thus aims at determining the shelf life of this local beverage during storage at variables temperature.

**Methodology:** Accelerated shelf life testing conducted at 4°C, 40°C, 50°C and 60°C were used to predict the shelf. A n-order mathematical expression were used to evaluate the shelf live and deterioration of “folere drink” an artisanal soft drink from northern Cameroon.

**Results and Conclusion:** The used Arrhenius model indicate that folere drink can be store for 258 day at 4±1°C, 100days at 9±3°C, 50 day at 15±5°C, 21 days at 30±2°C, and only 6 days at 25±3°C. The calculated Q<sub>10</sub> values were found to be in the range of 1.5-2.0, which is in the range for lipid oxidation in beverages reported by the literature.

Keywords: Shelf life; beverage; Africa.

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## **115: APPLICATION OF TERTIARY MODELS FOR PREDICTION OF *LISTERIA MONOCYTOGENES* GROWTH IN READY-TO-EAT MEALS SOLD IN SOUTH AFRICA**

Basirat Olaonipekun<sup>1</sup>, Ranil Coorey<sup>2</sup>, Elna Buys<sup>1</sup>

<sup>1</sup>University of Pretoria, Hatfield, South Africa. <sup>2</sup>Curtin University, Perth, Australia

**Introduction:** Despite the robust food safety management system used in the food industry, foodborne disease outbreaks such as listeriosis are still on the rise. Ready-to-eat

foods are widely consumed, posing food safety risk if food contaminated by *Listeria monocytogenes* is consumed. Application of predictive microbiology could assist the food industry to understand the behaviour of this organism in these categories food products to avert this problem.

**Methodology:** In this study, the presence of *Listeria* spp. in RTE beef lasagne (n=24) and egg noodles (n=24) stored for 6 days at  $5 \pm 1$  °C was evaluated for 6 months. A pack of each meal was also contaminated with *L. monocytogenes* ATCC 19115, using a sterile syringe, to obtain an initial concentration of  $6 \log_{10}$  cfu/g of the product in a challenge study. Literature review on existing predictive models for *L. monocytogenes* in RTE foods was also conducted, and some tertiary models (ComBase, FSSP, PMP and MicroHibro) were used to predict the growth of the pathogen and compared with the results from the challenge study.

**Results:** *Listeria* spp. was detected in all the beef lasagne and in 61% of the egg noodles. Strains of *L. seeligeri* and *L. monocytogenes* were detected in the RTE meals used in this study. Results of the challenge studies indicated that these food products support the growth of *L. monocytogenes* during chilled storage. Growth of *L. monocytogenes* predicted by ComBase, PMP, MicroHibro & FSSP in beef lasagne and egg noodles agreed with the observed growth from the challenge study with a fail-safe prediction. RTE meals supports the growth of *L. monocytogenes* under chilled storage indicating a high food safety risk to the public if the product is consumed.

**Conclusion and Relevance:** Predictive Microbiology is a field of Food Microbiology that can be explored by the food industry and food safety regulatory bodies, especially in Africa to assist in predicting the behaviour of foodborne pathogens during storage, thereby reducing the problem of food waste as result of product shelf life and at the same time protect public health.

**Keywords:** RTE meals; predictive tertiary models; *Listeria monocytogenes*; food safety; challenge study

## 91: TAKING ADVANTAGE OF THE *SALMONELLA* INACTIVATION IN TRADITIONAL DRY-FERMENTED SAUSAGES TO DEFINE A CORRECTIVE STORAGE.

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IRTA, Food Safety Programme, Monells, Spain

**Introduction:** The zero tolerance for *Salmonella* in ready-to-eat food poses a challenge for the meat industry, particularly in shelf-stable dry-fermented sausages (DFS). This study aimed to model the behaviour of *Salmonella* in the traditional catalan acid and low-acid DFS ("fuet") as a function of  $a_w$  and storage temperature in order to build a decision supporting tool to set a corrective storage leading to a safer product.

**Methodology:** The survival of *Salmonella* during the storage of acid (with starter culture) and low-acid (without starter culture) DFS was evaluated through challenge testing with

*Salmonella* inoculated in the meat batter at 6 Log cfu/g. Sausages were ripened to obtain 3 batches with different  $a_w$ . Storage was performed at 4, 8, 15 and 25°C. *Salmonella* was periodically enumerated on chromogenic agar. The Weibull model was fitted to Log counts data to estimate inactivation kinetic parameters. The impact of temperature and  $a_w$  was evaluated using a polynomial approach.

**Results:** *Salmonella* viability decreased during storage, the Weibull model satisfactorily fitted the data ( $R^2$  adjusted > 0.88). The effect of  $a_w$  and temperature was statistically significant, including a linear term for delta ( $\delta$ ) and a quadratic term for shape parameter ( $p$ ). Decreasing  $a_w$  and increasing temperature caused a decrease of  $\delta$ . A common  $p$  was obtained for acid and low-acid DFS, while  $\delta$  was product specific, indicating that starter culture affected the time required for the first Log reduction. Interestingly, a correlation was observed between the  $\delta$  values obtained for low-acid and acid DFS: the time to 1 log-inactivation in acid sausages was 50% (4°C) to 21% (25°C) shorter than in low-acid sausages. Technologically, a corrective storage at room temperature for 1 week or less could be implemented as a valid post-lethality treatment before fermented sausages are shipped to the market.

**Conclusion and Relevance:** Based on technologically and commercially feasible time-temperature corrective storage periods, the developed models can be used to define post-lethality treatments to enhance *Salmonella* inactivation in DFS. The impact of starter culture,  $a_w$  and storage temperature in *Salmonella* inactivation has been quantified and a decision-supporting tool to design a corrective storage as risk mitigation strategy before commercial launching has been developed.

**Keywords:** Foodborne pathogens; traditional dry-fermented sausages; modelling; corrective storage; non-thermal inactivation

## **6: PREDICTIVE MODELLING OF SURVIVAL *ESCHERICHIA COLI* ATCC 25922 UNDER DIFFERENT CONCENTRATIONS OF ARGAN OIL, SUGAR AND PEPTONE USING A RESPONSE SURFACE APPROACH**

Youssef Ezzaky, Mariem Zanzan, Fouad Achemchem

High Institute of Technology, Ibn Zohr University, Agadir, Morocco

**Introduction:** Almond, Honey and Argan Oil Spread, also known as Amlou, is a traditional recipe widely consumed in THE South of Morocco, and it is characterised by an artisanal production chain in which microbial contamination may be promoted. The aim of this study was to develop a model to predict the survival of *E. coli* ATCC 25922 in a medium matrix similar to Amlou as a function of argan oil, sugar concentration and peptone concentration.

**Methodology:** Doehlert matrix design and response surface methodology were used to find a relationship between factors (argan oil, sugar concentration and peptone concentration), and kinetics parameters: death rate (DR) and survival period (SP). The data generated within different conditions were fitted using Baranyi function. Then, separate quadratics models were developed with decimal logarithms of SP and DR as a

function of argan oil, sugar concentration and peptone concentrations to predict the evolution of *E. coli* in Amlou.

**Results:** Sugar concentration was found to have a significant effect on DR but not on SP; while argan oil and peptone concentration have shown a significant effect on SP, and not on DR. For the death rate, the coefficient of determination ( $R^2$ ), root means square error (RMSE), and standard error of prediction percentage (SEP) obtained were 0.95, 0.0095 and 22.22%, respectively. Although the  $R^2$  was 0.96, RMSE and SEP were 16.1 and 43.18%, a bit high for survival period.

**Conclusions and Relevance:** The statistical indexes obtained suggested that the models built to predict DR and SP of *E. coli* under experimental conditions fit well to the data. The results obtained in the present work could be used by producers of an artisanal product to predict *E. coli* response in foods having a similar composition.

Keywords: *Escherichia coli*; response surface; argan oil; sugar; protein

## 17: OPTIMISATION OF EXOPOLYSACCHARIDES PRODUCTION BY *LACTOCOCCUS LACTIS* A2 USING CENTRAL COMPOSITE DESIGN

Mariam ZANZAN<sup>1,2</sup>, Youssef Ezzaky<sup>1</sup>, Fatima Hamadi<sup>2</sup>, Fouad Achemchem<sup>1</sup>

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**Introduction:** *Lactococcus lactis* belongs to the lactic acid bacteria (LAB) group widely found in Mediterranean dairy products and generally considered as beneficial microorganisms. It is used to improve the quality of the food products by producing many beneficial substances. Exopolysaccharides (EPS) from LAB have attracted special attention as valuable bioactive because of their applications in industry. Bacterial EPS encompass a broad range of complex chemical structures and consequently exhibit different properties such as antioxidant, antibiofilm and antitumor activities. However, the low yield of EPS production severely hindered its application. The main objective of this work is to study the effect of medium components and culture conditions on cell growth and EPS production by *L. lactis* A2.

**Methodology:** Different values of maltose, yeast extract, pH, and cultivation time were evaluated in order to screen the factors influencing EPS production. The variables: maltose concentration, yeast extract, and pH were selected for the central composite design (CCD). Five levels ( $-\alpha$ , -1, 0, 1,  $+\alpha$ ) are shown and a  $2^3$  factorial design were conducted with six axial points (also called star points), and two replications of the center point (all the factors were at level 0), resulting in a total of 20 runs.

**Results:** The results showed that the optimum medium for the growth of *L. lactis* A2 (OD 620 nm) was obtained at the composition of maltose (60 g/L), yeast extract (45 g/L), and



initial pH (7). For EPS, maximum yields of 26.02 g/L were obtained at the optimal conditions of maltose (66 g/L), yeast extract (63 g/L), and initial pH (5.75). No correlation was shown between cell growth and EPS production.

**Conclusion and Relevance:** This study provided a high yield EPS by *L. lactis* A2, which suggests the use of the strain as a new source for the production of the bioactive polysaccharide. The results impart a reference for large-scale extraction of EPS by *L. lactis* A2 in industrial fermentation.

Keywords: Cell growth; Central Composite Design; maltose; yeast extract; bioactive

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## **Round Table: Assuring the Safety of Traditional Foods: A Scientific Contribution to Protecting our Cultural Heritage**

**Time: 17:40 - 18:40**

**Date: 18th September 2019**

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### **146: UNDERSTANDING MICROBIAL ECOLOGY AND INTERACTIONS IN TRADITIONAL FERMENTED FOODS: THE KEY ASPECTS**

Marciane Magnani

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Traditional fermented foods are linked to culture, traditions and history in several countries. The microbial population dynamics, interactions and distribution of microorganisms in traditional fermented foods support their identity while providing bio-tools for preservation and unique tastes and flavors. Scientific data on microbial ecology of traditional foods help to strengthen the quality and safety standards of these products, avoiding losses of heritage-foods around the world. One of the current challenges in microbiology of fermented foods is linking patterns of microbial diversity with parameters that cannot be easily explained by intrinsic factors. Important questions to be answered are: What defines the composition of a microbial community? When and how new microbial populations join or replace communities? What causes shifts in established microbial diversity over time?

Primarily biotic and abiotic selections are the basis of the microbial interaction in the imposed environment. Overall, by the consumption of nutrients and production of new compounds in the food matrix during fermentative processes, some populations are selected by inhibition of others, for example over ripening processes. However, random changes in the abundances of species within communities, as well as evolution of new species creating an unexpected diversity may mildly or drastically affect a fermented food product when new microorganisms can become dominant. In addition, small changes in

key aspects may create a condition where the unwanted microorganisms dominate instead of the desired ones. In this scenario, what can be really controlled? Can we trust the abiotic selection through traditional manufacture process, including the manipulation of salt, moisture, temperature, and pH? Metagenomics analysis has helped to understand mechanisms of microbial evolution, and to explain partially the microbial dynamic in fermented foods. Comparisons of genomic and phenotypic traits also have provided clear evidences of metabolic remodelling associated with adaptation to the fermentation environments and abiotic selection promoted by artisanal food producers. This overview will provide, based on various practical examples, the driver aspects on food microbial ecology without disregarding the human and geographically effects on microbial communities of safe traditional fermented foods.

Keywords: Microbial community; artisanal foods; metagenomics; microbial evolution

### **143: THE USE OF AUTOCHTHONOUS LACTIC ACID BACTERIA AS BIOPROTECTIVE CULTURES FOR ENHANCING THE SAFETY OF TRADITIONAL FOOD PRODUCTS: PERSPECTIVES AND LIMITATIONS**

Fouad Achemchem

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Traditional food products (TFP) are an integral part of the world's heritage and culture. Nevertheless, in many parts of the globe, producers of TFP still face the challenge to further improve the safety and the shelf-life of their products. Today's growing consumer demand for minimally processed or fresh food products with maximum safety and shelf life, while being free of chemical preservatives or containing only minimal amounts, is highlighting the challenge of TFP producers and leading them together with researchers in the agri-food sector to turn to alternative means of food preservation.

Biopreservation refers to extended storage time and enhanced safety of foods using natural microflora and/or their antimicrobial products. The inhibitory action of lactic acid bacteria (LAB) is basically due to the production of organic acids with the concomitant decrease of pH. In addition, some LAB strains produce antimicrobial peptides called bacteriocins. Nisin is the most common LAB bacteriocin, approved by the Food and Drug Administration and applied in over 50 countries around the world. LAB bacteriocins are applied in foods following different strategies: (i) inoculating directly LAB in foods as starter or adjacent cultures to produce the bacteriocins *in situ*; (ii) using the product previously fermented with the bacteriocin producing strain as an ingredient in the treatment of food; or (iii) adding the purified or semi-purified bacteriocin to the food system. This work will focus on recent developments and challenges towards the application of bacteriocins and bacteriocin-producing LAB as effective biopreservatives in the production of safe and healthy traditional food products.

Keywords: Biopreservation; fermentation; microbial competition; bacteriocins

#### **144: TRADITIONAL FOODS AS SOURCE OF MICROBIAL STRAINS TO BE EMPLOYED AS STARTER CULTURES: THE IMPORTANCE OF STRAIN ROBUSTNESS**

Anderson S. Sant'Ana

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Microorganisms have been widely used in industrial processes to obtain foods and bio-products. A key aspect for selecting a microbial strain for industrial use comprises its ability to withstand stressful conditions faced during processing. That feature is translated in terms of competition ability necessary for the microorganism to become dominant aiming to obtain a final product with the desired quality and safety. Microbial strains to be employed as starter cultures can present multifunctional properties such as ability to survive/growth under specific conditions, to produce useful compounds (vitamins, for instance), to release enzymes, to inhibit foodborne pathogens, and to present functional properties (probiotic claims), among others.

Several approaches can be employed in the selection of microbial strains for application as starter cultures for fermentation processes. In this study, a full characterisation of lactic acid bacteria isolated from Brazilian artisanal cheeses and the use of an evolutionary engineering approach to obtain a lactic acid strain with enhanced antimicrobial properties for cheese application will be described. The study highlights the potential of traditional foods as source of microorganisms with valuable biotechnological properties.

Keywords: Multifunctional properties; probiotics; Brazilian cheeses

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#### **133: THE ARTISANEFood PROJECT: NOVEL STRATEGIES TO ENSURE THE QUALITY OF TRADITIONAL FOODS PRODUCED IN THE MEDITERRANEAN**

Ursula Gonzales-Barron

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The Mediterranean region is known for being rich in traditional food products, and they constitute not only a vital part of the cultural heritage, but an important engine of many local economies. Thus, Mediterranean artisanal foods must be further valorised to improve their consumption and distribution; which, on the other hand, requires that local producers ensure the quality and safety of their products.

The objective of this project is to develop efficient bio-intervention strategies, enhanced process criteria, and an easy-to-use food safety decision support IT tool for participating artisanal food producers, aiming to the reduction and control of food-borne pathogens in 15 artisanal fermented foods of meat or dairy origin produced in Portugal, Spain, Italy,

France, Greece, Morocco, Tunisia and Algeria. The project will be developed through an integrated risk-based approach sustained by the concepts of (i) extensive tracking surveys in the artisanal food chains, in order to identify origin, routes of contamination, risk factors favouring pathogens' survival, and technological causes for lack of homogeneity in the quality/safety of end-products; (ii) biopreservation, whereby functional starter cultures and natural extracts will be assessed as extra hurdles to ensure safety and extend shelf-life; (iii) fate studies of pathogens, and (iv) risk process modelling, for the delineation of the most effective bio-interventions, optimisation of process variables and norms/standards, and design of quality monitoring tools.

A safety decision-support IT tool will be developed to enable artisanal producers to assess the lethality of their traditional and biopreservation-based manufacturing processes against pathogens. Uptake of the novel biopreservation technologies and quality monitoring schemes will bring about more efficient, harmonised and reliable food quality management systems of artisanal foods. Small regional businesses can thus become more competitive, and may reassuringly grow into companies of increased production and enlarged markets and exports opportunities. Efforts will be directed at establishing a Platform of Mediterranean Artisanal Food Producers, where food artisans - already in the project and others who wish to join - will keep up linkages with the ArtiSaneFood researchers in order to innovate on products and processes, and solve food safety issues through new collaborations and other ventures.

Keywords: Artisanal foods; biopreservation; starters; antimicrobial extracts; decision-support tool

## **150: MATHEMATICAL MODELLING AS A TOOL FOR ENSURING MICROBIOLOGICAL SAFETY OF TRADITIONAL FERMENTED FOODS**

Vasco Cadavez

CIMO Mountain Research Centre, Polytechnic Institute of Bragança, Bragança, Portugal

Artisanal fermented foods constitute not only important engines for regional economies, but part of the regional cultural heritage. The elaboration of these products, which have delighted us for centuries, results from the producers' knowledge and the invisible action of beneficial bacteria. However, spoilage and pathogenic bacteria are also lurking, and the producers must rely on a set of barriers that oppose their growth, namely: low contamination of raw materials, addition of salts (nitrites/nitrates), low pH, low  $A_w$ , smoking, etc. Thus, for further valorisation of traditional foods, it is necessary to ensure their quality and safety. This is where predictive microbiological models (PMM) come into play, making it possible to predict survivability of bacteria considering the environmental conditions (intrinsic and extrinsic) at which foods are subjected. The PMM are fed with laboratory data concerning the relevant microorganisms, challenged under the hurdles of salt, antimicrobials and microbial competition. Dynamic data is then used to estimate the parameters and validate the PMM.

Today, we are witnessing the growth of computing power and the development of web applications for predictive microbiology that are globally available. Thus, artisanal food

producers should benefit from these scientific and technological advances to inform their decisions on the safety of their products. This presentation will be focused on the theoretical concepts of the most appropriate PMM for fermented foods, as well as on the presentation of some practical cases of dynamic PMM applied to these products.

Keywords: Predictive microbiology models; fermentation; hurdles; artisanal foods

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## **74: DECISION-MAKING TOOLS FOR QUALITY AND SAFETY MANAGEMENT OF TRADITIONAL FOODS: THE CASE OF SPANISH-STYLE TABLE OLIVES**

Antonio Valero

Department of Food Science and Technology. University of Cordoba, Cordoba, Spain

Table olives are considered as one of the most representative traditional fermented vegetables in Mediterranean countries. Modernization and automation artisanal processes have been steadily implemented in the table olive sector since there are still industries lacking of food quality standards for process control. Implementation of quality certifications may serve to protect production methods as well as to increase quality assurance thus providing consumers' confidence in a more globalized market. In this sense, management of food productions is a fundamental aspect consisting on a decision-making process about the quality and safety of a food product coming from a certain lot. Probabilistic models can be integrated in decision-making tools allowing quantification of food safety and quality through the information retrieved from the table olive processing chain.

During the lecture, different applications of existing predictive modelling software on the table olive processing chain will be presented. In addition, a probabilistic model based on a weighing system will be shown, mimicking the production of Spanish-style table olives. This system is populated with physicochemical and microbiological parameters from table olive processing, together with information coming from the HACCP, existing hygiene plans in food industries, EU legislation, current hygienic -sanitary regulations and Codex recommendations. The results presented can be integrated within a software tool which will provide stakeholders with an easy-to-use, flexible and useful probabilistic decision-making scoring system for the Spanish-style table olive food sector. This allows determining the actual quality and safety levels, as well as records' management, and the assessment of the implementation of corrective measures throughout the processing chain. Furthermore, the approach can be extended to other olive varieties and elaboration methods including alternative treatments and steps being easily integrated within the quality management system of food industries.

Keywords: Table olives; probabilistic modelling; software tools; food safety; HACCP

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## Oral Session 5: Advances in Software and Databases Tools

**Time: 9:20 - 10:40**

**Date: 19th September 2019**

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### **113: FRESHINDEX - THE DYNAMIC "BEST-BEFORE" DATE**

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**Introduction:** Consumers are accustomed to throwing food away as soon as it reaches its “best-before” date or even before that, just to be safe. According to the European Commission up to 10% of the 88 million tonnes of EU food waste generated annually are linked to misinterpretation of “Best-Before” and “Use-by” dates. Any extension of the “best-before” date has the potential to reduce EU food waste. According to WARP a single additional day of shelf-life can reduce food waste by 5%. We believe that this can be realized by a dynamic expiry date, such as the one the FreshIndex project provides.

**Methodology:** The Project FreshIndex, aims at combining food modelling and big-data technologies to provide transparency to the food industry and their customers by a freshness related, dynamic best-before date and to validate this approach by field trials. The project consists of inter-disciplinary team of 5 partners and runs from 01/2018 to 12/2019.

**Results:** Predictive Modelling in food has made significant advances in the last decade. This scientific knowledge builds one cornerstone for our efforts. The other foundation is digital processing of food, food processing, packaging, storage and logistic data.

**Conclusion and Relevance:** In July and August 2019, the FreshIndex project will monitor the meat supply chain of METRO Germany. Product units will be traced digitally to the point of sale and the customer feedback recorded. The acquired data will be analyzed by a team of scientific and industry experts from multiple areas, such as microbiology and consumer studies and the results will be presented. We expected to prove that the sharing for data will make the food supply chain more transparent, reduce food waste and increase consumer satisfaction. We believe that this holds true for the exchange of scientific knowledge. METRO and tsenso want to reach out to the scientific community to extend the scope of the FreshIndex regarding products and methods. We are looking forward to interesting discussions, new ideas and future collaborations.

**Acknowledgements:** This project funded by the German Federal Ministry of Education and Research under grant number 03VNE2107.

Keywords: Shelf-life; industrial application; supply chain data; best-before date; food modelling

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## 20: FSSP v. 5.0 – A NEW SOFTWARE WITH PREDICTIVE MODELS FOR A RANGE OF DAIRY PRODUCTS

Paw Dalgaard<sup>1</sup>, Brian J. Cowan<sup>2</sup>, Veronica Martinez-Rios<sup>1</sup>

<sup>1</sup>National Food Institute (DTU Food), Technical University of Denmark, Kgs. Lyngby, Denmark.

<sup>2</sup>Anchor Lab KS, Copenhagen, Denmark

**Introduction:** Food Spoilage and Safety Predictor (FSSP) v. 4.0 from 2014 contained a growth and growth boundary model for *Listeria monocytogenes* in chilled seafood and meat products. This extensive model, including the effect of 12 environmental factors, has contributed to innovation, reformulation and documentation of safety for a wide range of seafood and meat recipes, including products with reduced sodium/salt content. The model has found little application for dairy products as its range of applicability was limited to  $\text{pH} \geq 5.6$  and did not include the inhibitory effect of dairy specific ingredients such as nisin and phosphate melting salts. FSSP v. 5.0 from 2019 has been developed to predict growth of *L. monocytogenes* in a range of dairy products.

**Methodology:** Three new *L. monocytogenes* growth and growth boundary models, including the effect of respectively four, six and 11 environmental factors, were developed and validated to include the inhibitor effect of  $\text{pH} \geq 4.6$ , mono-, di- and tri-phosphate melting salts and nisin in cheese. Separately, ten available *L. monocytogenes* models were evaluated with literature data for growth and survival of the pathogen in cheese (n=319). Two of these models were successfully validated to predict growth of *L. monocytogenes* in cheeses at constant and dynamic conditions including changes in temperature, pH, lactic acid concentration and water activity during storage.

**Results:** FSSP v. 5.0 includes models to predict the growth potential for *L. monocytogenes* in (i) chemically acidified and cream cheese with  $\text{pH} \geq 4.6$ ; (ii) processed cheese with mixtures of phosphate melting salts and residual concentrations of nisin A; (iii) smear cheese (DL-culture) and (iv) cheese in brine (O-culture). In addition previously developed models for growth of *L. monocytogenes*, lactic acid bacteria and psychrotolerant pseudomonads in cottage cheese and milk were included in FSSP v. 5.0.

**Conclusions and Relevance:** Extensive *L. monocytogenes* growth models specifically validated for dairy products and included in FSSP v. 5.0. This new software is likely to contribute to future product development, reformulation or risk assessment of dairy products in the same way that FSSP v. 4.0 has been used successfully for seafood and meat products (<http://fssp.food.dtu.dk>).

Keywords: Application software; *Listeria monocytogenes*; cheese; product development; risk assessment

## **22: DETECT: A DATABASE OF MICROBIAL RESPONSE WITH AN EMPHASIS ON VARIABILITY AND UNCERTAINTY**

Alberto Garre<sup>1</sup>, Heidy M.W. den Besten<sup>2</sup>, Marcel H. Zwietering<sup>2</sup>

<sup>1</sup>Universidad Politecnica de Cartagena, Cartagena, Spain. <sup>2</sup>Wageningen University, Wageningen, Netherlands

**Introduction:** Quantitative Microbial Risk Assessment (QMRA) is the preferred methodology for the evaluation of the risk associated to the consumption of food products. It is based on a quantitative description of the microbial response in each step of the food chain (growth and/or inactivation), based on mathematical modelling. In these models, the microbial response is characterised by model parameters that vary between bacterial strains, as well as with the environmental conditions.

**Methodology:** Experimental growth and inactivation experiments carried out by the Food Microbiology laboratory of the Wageningen University during the last decade have been retrieved. They have been compiled in a database that has been made available for the scientific community. A user-friendly interface has been implemented to increase its outreach.

**Results:** The database can contribute to future QMRA studies, informing especially the exposure assessment part. The microbial responses considered include microbial growth and inactivation under isothermal conditions for a range of bacterial strains (pathogenic and spoilage microorganisms). An emphasis has been made on addressing uncertainty and variability (reproduction and strain variability) by retrieving enough data to statistically quantify their contribution to the variance of the response.

**Conclusion and Relevance:** A database of microbial responses has been built. It has made available to the scientific community in Open Access in standard file format, so that it can be easily incorporated in future studies. The opportunity to extend it with additional data (internal and from other laboratories) has been considered, developing a software application to introduce new data. In future work, the database will be incorporated in a QMRA software tool. The quantification of microbial responses are essential for Quantitative Microbial Risk Assessment. The database developed enables an easy integration of this data in future studies by a broad range of researchers, including variability and uncertainty in the analysis.

**Keywords:** Exposure assessment; growth; inactivation; foodborne pathogens; variance analysis



## **102: MICROHIBRO AS A SOFTWARE TOOL FOR ESTABLISHING RISK-BASED MICROBIOLOGICAL CRITERIA IN FOODS**

Antonio Valero, Elena Carrasco, Guiomar Denisse Posada Izquierdo, Araceli Bolivar, Rosa M<sup>a</sup> García Gimeno, Gonzalo Zurera, Fernando Pérez Rodríguez

Department of Food Science and Technology. University of Cordoba, Cordoba, Spain

**Introduction:** MicroHibro is a predictive microbiology tool aimed to assess, quantitatively, the fate of potential pathogens in foods along the food chain and their impact on public health. The integration of risk assessment model outcomes in food testing performed by companies and food authorities is needed to develop suitable sampling plans and risk-based microbiological metrics. The objective of this work was to develop a specific module in MicroHibro able to make use of risk outputs in order to set and assess the performance of microbiological criteria and sampling plans.

**Methodology:** In this work, MicroHibro was upgraded by integrating a new module to determine and assess sampling plans, and risk metrics based on exposure assessment outputs generated by quantitative microbial risk model simulations. A demonstration of the performance of various sampling schemes is exemplified basing on certain underlying assumptions concerning the distribution of the microorganism within food batch and food unit. For this, several statistical approaches are considered (e.g. log-normal distribution, Poisson, random sampling).

**Results:** A decision supporting tool was implemented in the sampling plan and risk metrics module aimed to guide users in the selection of sampling plans in relation with Food Safety Objectives, and Performance Objectives. These metrics can be set on a prevalence or concentration basis and then assessed according to a certain safety margin or acceptability level chosen by user (i.e. percentage of non-compliant samples). Several sampling schemes can be tested as regards to the ability to detect non-conforming units and determine risk metric compliance.

**Conclusion and Relevance:** The performance of risk-based metrics and the establishment of microbiological criteria could help to identify critical steps along the food chain that influence on the final risk associated to a specific pathogen. MicroHibro is currently the only software integrating the outcomes provided by predictive microbiology models, risk assessment and sampling schemes of foodborne pathogens. The use of the MicroHibro software offers a flexible graphical user interface where models can be incorporated and/or evaluated as well as new sampling plans. This information may be used to evaluate the overall effectiveness of applied interventions by risk managers or food operators.

**Keywords:** Predictive modelling software; statistical distributions; risk-based metrics; foodborne pathogens; risk assessment

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## 134: PATHOGENS-IN-FOODS: A WEB APPLICATION TO ACCESS AND META-ANALYSE OCCURRENCE DATA OF MICROBIAL HAZARDS IN FOODS

Vasco Cadavez<sup>1</sup>, Pauline Kooh<sup>2</sup>, Moez Sanaa<sup>2</sup>, Ursula Gonzales-Barron<sup>1</sup>

<sup>1</sup>CIMO Mountain Research Centre, Polytechnic Institute of Bragança, Bragança, Portugal. <sup>2</sup>French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Maisons-Alfort, France

**Introduction:** In the literature, there are many investigations addressing the quantification of the occurrence of biological hazards in foods. Having access to this information has become relevant in risk assessment and management of foodborne pathogens by both researchers and food safety authorities. Nevertheless, this information is dispersed, disharmonised and not always accessible. Thus, the objective of this study is to bring together, under a harmonised arrangement, all good-quality data on the occurrence of pathogens in foods sold in Europe. Thus, the work was carried out in two stages: (i) first, a systematic review and data extraction from available literature on the occurrence of 14 biological hazards according to food matrix category; and (ii) second, the construction of a relational database and a dynamic-reporting web application that facilitate data access and retrieval according to bacterium, food class, country or any other variable, with the ability to produce summary statistics, charts and meta-analysis according to selections made by the user.

**Methodology:** Systematic literature searches were conducted for *Salmonella*, *Campylobacter*, Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Toxoplasma gondii*, norovirus, Hepatitis A virus, Hepatitis E virus, *Cryptosporidium* and *Giardia duodenalis*; and after screening for methodological quality, data were carefully extracted into a harmonised arrangement of primary study characteristics, food characteristics and stage within the food chain, microbiological methods, prevalence/enumeration results, and mechanism for handling substitution. References are managed in JabRef with direct links to the sources in PDF.

**Results:** So far, the Pathogens-In-Foods database (<https://pathogensinfood.esa.ipb.pt/web/index.php>) contains ~6000 observations extracted from 734 studies reporting on microbial pathogens, 71 on parasites and 172 on viruses. The database was created using MySQL. The management of the database, tables, columns, relations, indexes, users and permissions was developed using PHP language, while the interface for dynamic reporting was done through the *shiny* and *flexdashboard* packages implemented in R. Users can generate summary tables, charts, boxplots, trend graphs and meta-analysis of their data selections.

**Conclusion and Relevance:** Pathogens-In-Foods is intended to provide an easier and usable interface for researchers and other end-users to retrieve occurrence data of pathogens, and quickly obtain descriptive statistics, graphs and meta-analysis. Pathogens-In-Foods is being updated and improved.

Keywords: Web application; database; dynamic report; meta-analysis; Shiny

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### **63: HOW MANY SAMPLES SHALL I TAKE? AN ANSWER BASED ON NUMERICAL SIMULATIONS AND INFORMATION THEORY**

Alberto Garre<sup>1</sup>, José L. Peñalver<sup>1</sup>, Marta Clemente<sup>1</sup>, Paula M. Periago<sup>1</sup>, Pablo S. Fernández<sup>1</sup>, Arantxa Aznar<sup>1</sup>, Arturo Esnoz<sup>1</sup>, Alfredo Palop<sup>1</sup>, Jose A. Egea<sup>2</sup>

<sup>1</sup>Universidad Politécnica de Cartagena, Cartagena, Spain. <sup>2</sup>Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Murcia, Spain

**Introduction:** Predictive modelling has during the last years gained status as a core tool for food industries and regulatory agencies. However, models used in food science usually have model parameters that have to be estimated based on experimental data. Due to experimental uncertainty and variability, these parameters cannot be known with infinite precision. Instead, they must be reported with a measure of uncertainty (e.g. a standard deviation). It is believed that an increase in the number of sampling points will increase precision, but there is, to date, no way to predict whether an experimental design is likely to attain the desired precision. The objective of this research was to develop tools that aid in the design of microbial inactivation experiments in order to improve the precision of the parameters obtained from them.

**Methodology:** Two complementary approaches to estimate the standard deviation of model parameters before conducting any simulations are presented. The first is a resampling technique based on Monte Carlo simulations. The second is based on the properties of the Fisher Information Matrix, which can be used as an estimator of the size of the confidence ellipsoids.

**Results:** The methodologies presented are applied to estimate the standard deviation of the model parameters characterizing microbial inactivation (D-value and z-value) for isothermal and dynamic inactivation. They are used to compare the precision of different experimental designs (number of sampling points, shape of the temperature profile, optimal against uniform designs).

**Conclusion and Relevance:** The computational methodologies proposed can aid experimental design, estimating whether an experimental design is likely to result in the desired precision in parameter estimates. Moreover, the case studies analysed demonstrate that the temperature profile impact parameter precision and the superiority of optimal with respect to uniform designs. Indeed, increasing the number of sampling points in uniform designs does not ensure more precision. Parameter estimation is a core problem in predictive microbiology. The computational techniques presented here can aid experimental design, estimating the precision attained with an experimental design. This would enable more efficient experimental workflows.

Keywords: Numerical simulation; information theory; experimental design; microbial inactivation kinetics

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## Oral Session 6: Meta-Analysis Protocols and Applications

Time: 11:10 - 12:30

Date: 19th September 2019

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### 40: A VARIETY OF VEGETATIVE BACTERIAL PATHOGENS SEEMS TO HAVE SIMILAR THERMAL RESISTANCE, AS BETWEEN-SPECIES VARIABILITY IS MUCH LOWER THAN WITHIN-SPECIES VARIABILITY

J. Hein M. van Lieverloo<sup>1,2</sup>, Marjon H.J. Wells-Bennik<sup>3</sup>, Heidy M.W. den Besten<sup>4</sup>, Marcel H. Zwietering<sup>4</sup>

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<sup>2</sup>Viaeterna, Rosmalen, Netherlands. <sup>3</sup>NIZO, Ede, Netherlands. <sup>4</sup>Food Microbiology, Wageningen University, Wageningen, Netherlands

**Introduction:** Meta-models of time-temperature inactivation data of vegetative pathogenic bacteria from literature show large overall variability (Van Asselt & Zwietering, 2006). Observed variability can be reduced by adjusting for the effects of especially water activity, pH and experimental conditions using multiple regression analysis (Van Lieverloo et al., 2013a, 2013b, 2017). The hypothesis of this study was that the vegetative cells of a wide variety of pathogenic bacteria species have similar heat inactivation kinetics: their within-species variability is larger than between-species variability.

**Methodology:** This study is based on a model for heat inactivation of *Salmonella enterica* (514 data sets on media, juices, peanut butter and chocolate, 26 serovars): logD vs. temperature alone:  $R^2 = 0.003$ ,  $z = 160$  °C. logD vs. temperature, water activity, pH and sugar content (multiple regression analysis):  $R^2 = 0.79$ ,  $z = 5.9$  °C, s.e. = 0.42, Van Lieverloo *et al.*, ICPMF, 2017). Literature data on the logD of vegetative cells of nine other pathogenic bacteria species since 1965 were compared to those predicted with the *Salmonella* model. When specific data on water activity ( $a_w$ ), pH and sugar content were not supplied, these were supplemented from generic sources.

**Results:** The mean and within-species variability of thermal resistance *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica*, *Cronobacter* spp. and vegetative cells of *Clostridium perfringens* are similar to those of *Salmonella enterica* (within 95%-prediction interval). For *Staphylococcus aureus* some higher thermal resistance data were found (many were similar), while for *Vibrio parahaemolyticus* and *Vibrio vulnificus* most were similar, but < 50 °C some lower resistance data were found.

**Conclusion and Relevance:** In a meta-analysis of literature data, a basic inactivation model for *Salmonella enterica* was developed, only adjusting for water activity, pH and

sugar content. A variety of nine other pathogenic bacterial species, both Gram-positive and Gram-negative, spore-forming or not, show similar mean thermal resistance and within-species variability. Further research may show that one model, using the same z-values, reference temperatures and prediction intervals for temperature, water activity, pH and other conditions, suffices for the design of thermal inactivation of vegetative cells of many bacterial pathogens.

Keywords: Heat inactivation; bacteria; multiple regression; water activity; statistics

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## 149: META-REGRESSION MODELS DESCRIBING THE EFFECTS OF ESSENTIAL OILS AND ADDED LACTIC ACID BACTERIA ON *L. MONOCYTOGENES* INACTIVATION IN CHEESE

Beatriz Nunes Silva<sup>1,2</sup>, Vasco Cadavez<sup>1</sup>, José António Teixeira<sup>2</sup>, Ursula Gonzales-Barron<sup>1</sup>

<sup>1</sup>CIMO Mountain Research Centre, School of Agriculture, Polytechnic Institute of Braganza, Braganza, Portugal. <sup>2</sup>CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal

**Introduction:** Biopreservatives such as plant-based antimicrobials and bacteriocinogenic starter cultures have been proposed as hurdles to increase microbiological safety of cheeses. In this study, meta-regression models were built to summarise the effectiveness of essential oils (EO) and added lactic acid bacteria (LAB) on *L. monocytogenes* (LM) inactivation in cheese, and to evaluate other affecting factors and possible interactions.

**Methodology:** Suitable primary studies were identified through systematic literature search. From twenty-three studies reporting data on LAB and EO effects on LM counts in cheese, 282 and 322 entries were collected, respectively. The following information was obtained: study ID, antimicrobial class (EO or LAB) and name, LM mean log reduction, storage temperature, exposure time, application type (in milk or on cheese surface), antimicrobial concentration and pathogen inoculum level. Then, mixed-effects linear models with weights were separately adjusted to the LAB and EO data sets, with exposure time and antimicrobial concentration as nested fixed effects in application type.

**Results:** The results of the meta-regression model for the LAB data set revealed the significant impact of the application type ( $p=0.001$ ), pathogen inoculum level ( $p<.0001$ ) and storage temperature ( $p=0.001$ ) on LM inactivation in cheese; while LAB concentration applied showed no significant effect ( $p=0.3688$ ). An interaction between exposure time and type of application was also observed ( $p<.0001$ ), meaning that the treatment duration, for the same LM reduction, depends if the antimicrobial is added to the milk or to cheese surface.

Regarding the EO-LM meta-model, the results showed, again, the significant effects of pathogen inoculum level ( $p<.0001$ ), storage temperature ( $p=0.0004$ ) and application type ( $p<.0001$ ), the latter meaning that microbial reduction is faster when the EO is added to the milk ( $b=0.488$ ), rather than onto the surface ( $b=0.334$ ). Interactions between application type and exposure time ( $p<.0001$ ) were also observed. Overall, the anti-listerial effect of EOs depends on its origin, yet, seemingly, those extracted from mint,

oregano, salvia and basil present the greatest bactericidal effects in cheese matrix, as per analysis of random-effect marginal intercepts and concentration slopes.

**Conclusion and Relevance:** Globally, the effect of antimicrobials on LM reduction differs when applied in milk or on the cheese surface, and it is affected by antimicrobial concentration, storage temperature and time. The fact that pathogen's inoculum level consistently appeared as a moderator driving the measured reductions should be further investigated.

**Keywords:** Biopreservation; meta-analysis; starter culture; antilisterial activity; mixed-effects linear model

## **99: DEVELOPMENT OF A GENERAL MODEL TO DESCRIBE *SALMONELLA* spp. GROWTH IN CHICKEN MEAT SUBJECTED TO DIFFERENT TEMPERATURE PROFILES**

Tatiane Milkiewicz<sup>1</sup>, Vinícius Badia<sup>1</sup>, Daniel Longhi<sup>2</sup>, Denise Stolf<sup>3</sup>, Alessandro Galvão<sup>1</sup>, Weber Robazza<sup>1</sup>

<sup>1</sup>Santa Catarina State University, Pinhalzinho/SC, Brazil. <sup>2</sup>Paraná Federal University, Jandaia do Sul/PR, Brazil. <sup>3</sup>UCEFF University, Chapecó/SC, Brazil

**Introduction:** In this study, a general model to predict *Salmonella* growth in chicken meat subjected to both isothermal and non-isothermal temperature profiles was developed and validated.

**Methodology:** To accomplish this task, three different primary (Baranyi-Roberts, Huang, and Robazza et al.) and three different secondary (Ratkowsky et al., Huang, and Rosso et al.) models were taken from the literature and tested against 250 isothermal datasets regarding *Salmonella* growth in chicken meat selected from Combase. The following statistical indices were considered to compare the models: Akaike Information Criterion, Bayesian Information Criterion, Accuracy Factor, Bias Factor, Mean Absolute Error, and Root Mean Square Error. The resulting model comprising the primary and secondary models that provided the best fit to the selected data sets was numerically integrated and validated against 4 non-isothermal temperature profiles of *Salmonella* growth in chicken meat also selected from the literature. After the validation, a few simulations were conducted to evaluate the influence of small temperature shifts corresponding to situations of temperature abuse in chicken meat on the *Salmonella* population. Two empirical equations that predict the time to a 1-log and a 2-log increase in the bacterial loads in terms of the amplitude of the temperature shift were obtained.

**Results:** The primary model of Huang was considered to provide the best fit, and the Ratkowsky et al. and Huang secondary models were considered to be the best secondary models to describe the experimental data. The minimum temperature for *Salmonella* growth was empirically estimated to be approximately 6 °C. In addition, it was observed that the time to 1-log and 2-log increases were of approximately 96.7 and 188.0 hours respectively in a scenario corresponding to a shift of 0.5 °C in relation to an initial

temperature of 6 °C and decreased hyperbolically with the increase in the temperature shift.

**Conclusion and Relevance:** Because the chicken products are often subjected to small shifts in the temperature during processing, storage, and transportation until reaching the final consumer, these results should be explicitly considered in the development of risk assessment of chicken products with regard to contamination with *Salmonella*.

Keywords: *Salmonella* spp; chicken meat; modelling; temperature; shift

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## **60: SENSORY EVALUATION OF SEAFOOD FRESHNESS USING THE QUALITY INDEX METHOD: A SYSTEMATIC REVIEW USING META-ANALYSIS**

Eduardo Esteves<sup>1,2</sup>, Jaime Aníbal<sup>1,3</sup>

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**Introduction:** The quality index method (QIM) is a leading method of assessing the freshness (and thus quality) of seafood that is based on relatively few sensory quality parameters considered relevant. These characteristics are scored using a 0 to 3 demerit points' scale, the sum of which is designated the quality index (QI) and quantifies the specimens' lack of freshness. The linear relationship between QI and storage time allows for the estimation of remaining shelf-life. Moreover, QIM is deemed species-specific.

**Methodology:** In this work, meta-analysis using random/mixed-effects models of >60 primary studies was carried out to examine the variation of the QIM schemes among "commercial" categories of seafood (fatty and lean fish, crustaceans and cephalopods), as stipulated in the European Union's Council Regulation (EC) No. 2406/96, as well as reported storage temperatures, mode of preparation (whole, filleted, beheaded, gutted, precooked, peeled) and/or methods of preservation (water, air, vacuum and modified atmosphere and ice) to test QIM schemes' species-specificity and examine the relative effect of the above mentioned factors on QIM's species-specificity.

**Results:** No evidence supporting grouping of QIM schemes by category of seafood and/or preservation method was found even when potentially important or extraneous factors, e.g. storage procedure and temperature or preparation mode, were considered.

**Conclusion and Relevance:** Seemingly, the results corroborate method's species-specificity. Findings and methodological procedures are discussed in light of published research.

Keywords: Quality Index Method; meta-analysis; EU regulation; seafood freshness

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## 127: META-ANALYSIS OF RISK FACTORS FOR TWO FOODBORNE VIRAL DISEASES: NOROVIRUS AND HEPATITIS E VIRUS

Anne Thebault<sup>1</sup>, Ursula Gonzales-Barron<sup>2</sup>, Vasco Cadavez<sup>2</sup>, Pauline Kooh<sup>1</sup>, Moez Sanaa<sup>1</sup>, Nicole Pavio<sup>3</sup>

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**Introduction:** Norovirus and Hepatitis E virus are two of the three main viral foodborne pathogens. Norovirus is the main source of human gastroenteritis, and hepatitis E the most important foodborne zoonotic viral disease for humans in Western countries. The objective of this work was to study the associations between risk factors and sporadic cases by meta-analysing outcomes from case-control and cohort studies.

**Methodology:** Suitable scientific articles were identified through systematic literature search, and subjected to a methodological quality assessment. From each study, odds-ratio (OR) measures were extracted/calculated, as well as study characteristics such as population type, design, type of model and risk factor hierarchy. Mixed-effects meta-analysis models were adjusted by population type to appropriate data partitions.

**Results:** Hepatitis E cases were mainly defined with serological exam associated or not with symptoms, while norovirus defined by molecular identification in stools associated or not with symptoms. For Hepatitis E, a total of 78 relevant primary studies provided 582 ORs. Norovirus cases were investigated in only 14 studies providing 105 ORs. Hepatitis E with serological definition was associated with: blood transfusion, lack of hygiene, drink water or farm occupation, and food products such as meat (in particular pig products, game meat, undercooked pork meat), seafood, dairy, drink water and produce. In contrast, Hepatitis E cases were only associated with meat products such as pig meat, other red meats and processed meat. For norovirus, person-to-person transmission, eating undercooked, outside or in food markets, and shellfish consumption were significant risk factors, while handwashing was identified as a protective factor.

**Conclusion and Relevance:** This meta-analysis identified the most common transmission pathways for Hepatitis E and norovirus, and should provide epidemiological information needed by health-care professionals and policy-makers. Sporadic cases of norovirus were under-studied, probably because of the high level of information provided by outbreaks. Hepatitis E sporadic cases could be more investigated. The definition of risk factors was sometimes too large for management purposes, such as seafood for shellfish, and should include cooking practices. Geographical differences, in relation to strains involved could be better explored, with larger data sets.

**Keywords:** Case-control study; cohort study; odds-ratio; sources



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## 128: META-ANALYSIS OF RISK FACTORS FOR SPORADIC INFECTIONS CAUSED BY TWO FOODBORNE PARASITES: *TOXOPLASMA GONDII* AND *GIARDA INTESTINALIS*

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**Introduction:** *Toxoplasma gondii* and *Giardia intestinalis* are two of the three main parasitic foodborne pathogens. The objective of this work was to sum up evidence on the associations between risk factors and sporadic cases by meta-analysing outcomes from case-control and cohort studies for those two different pathogens.

**Methodology:** Suitable scientific articles were identified through systematic literature search, and subjected to a methodological quality assessment. From each study, odds-ratio (OR) measures were extracted or calculated, as well as study characteristics such as population type, design, type of model and risk factor hierarchy. Mixed-effects meta-analysis models were adjusted on ln(OR) by population type to appropriate data partitions relative to risk factors.

**Results:** *Toxoplasma* cases were mainly defined with serological exams and *Giardia* cases by stool sample analysis. For toxoplasmosis, a total of 200 relevant primary studies provided 2050 ORs, while giardiasis was investigated in 72 studies, which provided 736 ORs. As an example of results, *Toxoplasma* seropositivity in pregnant women was associated with: immunocompromising disease (pooled OR=2.094; 95% CI: 1.497 – 2.930), consumption of undercooked beef (OR=2.052; 95% CI: 1.576 – 2.672), poor handling of foods (OR=2.00; 95% CI: 1.958 – 2.504), and contact with cats (OR=1.631; 95% CI: 1.445 – 1.841). For giardiasis, the main risk factors of transmission for children were: person-to-person contagion (OR=3.404; 95% CI: 1.873-6.187); contact with animals (i.e., farm animals OR=1.550; 95% CI 1.195-2.021); contact with different types of contaminated environments (i.e, exposure to waste water OR=1.702; 95% CI: 1.416 - 2.047); drinking water (OR=1.746; 95%CI: 1.397 - 2.182) and produce consumption (OR=2.192; 95% CI: 1.465 - 3.278). Breastfeeding was found to have a protective effect against acquiring giardiasis (OR=0.638; 95% CI: 0.499 - 0.817).

**Conclusion and Relevance:** This meta-analysis identified the most common transmission pathways for toxoplasmosis and giardiasis, and should provide epidemiological information needed by health-care professionals and policy-makers. This comparison shows also specific and common issues between *Giardia* and *Toxoplasma* related to the type of epidemiological studies, population at risk, risk factors definition and main geographical areas of concern for each pathogen.

**Keywords:** Case-control study; cohort study; source attribution; toxoplasmosis; giardiasis

## 129: META-ANALYSIS OF RISK FACTORS FOR SPORADIC CAMPYLOBACTERIOSIS AND LISTERIOSIS

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**Introduction:** *Campylobacter* spp. is a widespread and important cause of human illness worldwide. Disease is frequently associated with foodborne transmission, but other routes of exposure are also recognised. Human listeriosis is a rare but serious foodborne disease, with high morbidity and mortality in vulnerable populations (e.g., pregnant women and the unborn, newborns, the elderly, and the immunocompromised). Most cases are foodborne but identifying specific food vehicles can be problematic due the ubiquitous nature of the pathogen. The objective of this work was to sum up evidence on the associations between risk factors and sporadic cases of campylobacteriosis and listeriosis by meta-analysing outcomes from case-control studies for those two pathogens.

**Methodology:** Suitable scientific articles were identified through systematic literature search, and subjected to a methodological quality assessment. From each study, odds-ratio (OR) measures were extracted or calculated, as well as study characteristics such as population type, design, type of model and risk factor hierarchy. Mixed-effects meta-analyses models were adjusted on ln(OR) by population type to appropriate data partitions related to risk factors.

**Results:** Sporadic campylobacteriosis was investigated in 71 primary studies providing 1336 ORs. In the mixed population, the highest significant associations were found for international travel, recent use of anti-acids, occupational exposure to animals/carcasses, contact with farm animals, consumption of meat of non-specified origin and raw milk. In the children population, the main risk factors were drinking untreated water, exposure to recreational water, exposure to farm/rural environment, contact with farm animals, person-to-person contagion and consumption of raw milk, and meat of non-specified origin. It was estimated that breastfeeding exerts a protective effect ( $p < 0.05$ ).

For *Listeria monocytogenes*, a total of 12 primary studies provided 227 ORs. According to this meta-analysis study, the most important risk factors for acquiring listeriosis in the susceptible population were: suffering from a chronic disease or other medical conditions, and the recent use of anti-acids. Among the food vehicles, the highest associations were found for seafood and dairy products.

**Conclusion and Relevance:** This meta-analysis identified the most common transmission pathways for campylobacteriosis and listeriosis, and should provide epidemiological information needed by health-care professionals and policy-makers.

Keywords: Case-control study; odds-ratio; source attribution; *Campylobacter* spp.; *Listeria monocytogenes*

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## Oral Session 7: Advances in Risk assessment Methods and Integration of Omics Techniques

Time: 14:40 - 16:10

Date: 19th September 2019

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### 4: INCORPORATION OF WHOLE-GENOME-SEQUENCING DATA IN EXPOSURE ASSESSMENT: MACHINE LEARNING AND NETWORK-ANALYSIS APPROACHES

Patrick Murigu Kamau Njage<sup>1</sup>, Pimlapas Leekitcharoenphon<sup>1</sup>, Ana-Rita Bastos Rebelo<sup>1</sup>, Lisbeth Truelstrup Hansen<sup>1</sup>, Rene Hendriksen<sup>1</sup>, Matteo Bersanelli<sup>2</sup>, Ettore Mosca<sup>3</sup>, Tine Hald<sup>1</sup>

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**Introduction:** Despite gains in accurate subtyping of microbial hazards using high-throughput DNA sequencing technologies, the incorporation of this data in exposure assessment during MRA remains scantily explored. We propose incorporating machine learning (ML) and network-diffusion based analysis with a case of *Listeria monocytogenes* for exposure assessment considering response to the four stress conditions: desiccation, salt concentration, pH and low temperature.

**Methodology:** Histograms of maximum growth rates of *Listeria* for the four stress conditions indicated multi-modality which cannot easily be described by standard distributions. Inference for these mixed populations of *Listeria* stress tolerance was made using finite mixture models to reveal underlying categories, mean maximum growth rate for each, proportion of the population of isolates in each particular tolerance category and to classify each of the isolates into a category of the mixture.

**Results:** This yielded categories such as highly susceptible, susceptible, tolerant and highly tolerant for acid stress. With such categories in mind, further interest was in making predictions about the phenotype category using the genotype. Total of 7348 accessory genes in amino acid sequences were used as model input to predict and differentiate each of the stress categories using supervised ML models. A matrix of percent similarity between accessory genes and the *Listeria* genomes was generated and subsequently used as input for ML. Six ML algorithms were evaluated for their prediction accuracy. Random forest was chosen as appropriate model for the cold (Accuracy: 96%; CI: 90-99%), salt (Accuracy: 86%; CI: 79-92%) and desiccation stress (Accuracy: 91%; CI: 85-96%) responses although it performed as good as other choices for some stress responses.

Support vector machine (Accuracy: 88%; CI: 81-94%) was the best model in prediction of acid tolerance. Top genes contributing to the predictions were also selected and may be included in reduced predictive laboratory assays and models. Network diffusion based analysis of genomic profiles enabled further analysis of areas of the protein-protein interaction with specific role in shift towards susceptibility, tolerance or overall shift. Specific contribution was clear for desiccation and salt stress phenotypes.

**Conclusions and Relevance:** Next steps towards incorporation into microbial risk assessment, validation and interpretation of completed modelling outputs will be presented.

**Keywords:** Exposure assessment, *Listeria monocytogenes*, machine learning, network analysis, whole genome sequencing

## 16: QUANTITATIVE ASSESSMENT OF MICROBIOLOGICAL RISKS DUE TO RED MEAT CONSUMPTION IN FRANCE

Juliana De Oliveira Mota<sup>1</sup>, Fabrice Pierre<sup>2</sup>, Sandrine Guillou<sup>1</sup>, Jeanne-Marie Membré<sup>1</sup>

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**Introduction:** Red meat is a major food component associated with foodborne illnesses due to microbiological hazards, as reported by European Centre for Disease Prevention and Control and European Food Safety Authority (EFSA). The objective of this study was to quantify the foodborne illnesses and subsequent burden of diseases (expressed in Disability Adjusted Life Years, DALY) due to the eight main microbiological hazards encountered in red meat in France.

**Methodology:** Data in the literature and official organizations portal such as World Health Organization (WHO), Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses), l'institut national de la statistique et des études économiques (Insee) and EFSA, were aggregated to identify and characterise the main microbiological hazards associated with red meat consumption in France. Then, the associated number of foodborne illnesses and the subsequent burden of diseases, expressed in DALY were estimated.

**Results:** In France, per year, it was estimated 520 [95% CI = 300-890] illnesses per 100,000 inhabitants due to red meat consumption, corresponding to 11 [95% CI = 7-17] DALYs per 100,000 population. In terms of human cases, *Campylobacter* spp. was ranked at the top with 180 [95% CI = 60-400] cases per 100,000 population per year, while in term of DALYs, the major contributor from red meat consumption was non-typhoidal *Salmonella* enterica with 4 [95% CI = 1-8] DALYs per 100,000 population per year. The most severe foodborne hazard was found to be *Brucella* spp. accounting for 0.43 [95% CI = 0.02-1.56] DALY per case, although its incidence remained low.

**Conclusions and Relevance:** This study provided an overview of the current microbiological risks due to red meat consumption in France. Results could be

implemented in a more global risk-benefit assessment associated with red meat consumption, beside nutritional and chemical risks and nutritional benefit.

Keywords: Foodborne illnesses; red meat; public health; DALY

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## **61: MODELLING THE TRANSMISSION OF ANTIMICROBIAL RESISTANCE BETWEEN ANIMALS AND HUMANS USING METAGENOMICS**

Ana Sofia Ribeiro Duarte, Timo Röder, Patrick Munk, Thomas N. Petersen, Tine Hald

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The food safety community is currently embracing metagenomics, and assessing its potential and suitability in several areas, including source-attribution. Source-attribution is among the array of modelling approaches that support food safety management. Microbial subtyping based source-attribution allows to infer the source of transmission of foodborne pathogens to humans with foodborne illness, through the comparison of pathogen subtypes observed in different food-producing animal reservoirs with those observed among human cases. It includes population genetic models and frequency-based models.

Compared to whole genome sequencing, metagenomics offers the advantage of unveiling the genetic content of the total microbiota in a food product, in the gut of humans and in the gut of animal reservoirs. This possibility is particularly advantageous when the aim is to attribute not the infections by a particular pathogen, but the occurrence of antimicrobial resistance (AMR) genetic determinants in the human gut. This attribution is relevant because some AMR genes (ARGs) may be considered a hazard *per se*, due to their ability to be horizontally transferred between distinct bacterial species within the human gut. With metagenomics, it is possible to investigate the span of ARGs that characterize the resistome of different animal reservoirs, and those that characterize the resistome of food consumers. There is however the need to develop an appropriate source-attribution method to assess the links between those resistomes. Population genetic models are most appropriate to isolate-based typing data, and existing frequency-based models have been often overwhelmed by the dimensionality in next generation sequencing data. Hence, there is a need for new modelling approaches.

We hypothesized that the abundance of particular ARGs is associated with specific animal reservoirs, and explored this association using a supervised classification machine learning algorithm, random forest. We developed two model versions - one with resistome data from animal reservoirs only, and another including resistome data from a cohort of healthy humans, thereby considering “human” as a source. Both models were used to predict the source of human resistomes, expressed as the probability of a human resistome originating from different reservoirs.

Keywords: Metagenomics; source-attribution; antimicrobial resistance; resistome; machine learning

## **117: A FRAMEWORK TO EVALUATE THE IMPACT OF FOOD INTAKE SHIFTS ON RISK OF ILLNESS USING A CASE STUDY WITH INFANT CEREAL**

Sofia Santillana Farakos<sup>1</sup>, Regis Pouillot<sup>2</sup>, Judith Spungen<sup>1</sup>, Brenna Flannery<sup>1</sup>, Laurie Dolan<sup>1</sup>, Jane Van Doren<sup>1</sup>

<sup>1</sup>US FDA, College Park, USA. <sup>2</sup>Independent Consultor, Buenos Aires, Argentina

**Introduction:** We developed a framework to provide decision makers with a multi-faceted evaluation of the impact of dietary shifts on risk of illness in the U.S. population.

**Methodology:** We collected representative data on prevalence and concentration of inorganic arsenic and aflatoxin in infant rice and oat cereal. Exposure to these contaminants through consumption and risk of illness from cancer were assessed per consumer based on data from the National Health and Nutrition Examination Survey and published dose-response and related data. The expected number of additional cases of illness and disability-adjusted life years (DALYs) for the U.S. population were estimated. The public health impact of shifts in consumption from one product to the other considered marginal and joint consumption and characterised uncertainty arising from estimates of contaminant concentrations, bioavailability and dose-response models. Monte Carlo simulations were developed in R and a Shiny app was created.

**Results:** Based on current consumption of infant rice and oat cereal, the estimated additional DALY for the total US population from inorganic arsenic and aflatoxin is 4,600 (CI 90% [370; 8,400]). If all consumers shift their consumption (maintaining equivalent servings) to only infant rice or only infant oat cereal, the estimated DALY increases to 1.4 and decreases to 0.4 relative to the baseline, respectively. Changes in contaminant concentrations or percent consumers also significantly impact risk. Uncertainty in risk estimates is primarily driven by the dose-response models for this case study. These results support previous advice on varying grain intake in children.

**Conclusion and Relevance:** The current risk-risk framework can provide decision makers with a nuanced understanding of the impact of consumption shifts on public health and reveal parameters that drive predicted changes in public health. The Shiny app provides a real-time visualisation tool to facilitate understanding and allow direct query by decision makers. The case study showcases applicability of the framework for a wide range of food safety and nutrition questions.

**Keywords:** Consumption shift; risk of illness; risk-risk analysis; infant cereal; Shiny

## 50: IMPACT OF CONSUMER BEHAVIOUR ON RISK TO HUMAN HEALTH FROM DRINKING RAW MILK

Sarah Pirikahu<sup>1</sup>, Tanya Soboleva<sup>2</sup>, Beverley Horn<sup>1</sup>, Peter Cressey<sup>1</sup>

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**Introduction:** New Zealand's Institute of Environmental Science and Research in conjunction with the Ministry of Primary Industries (MPI) has conducted a quantitative microbiological risk assessment (QMRA) of the risk to human health from drinking raw milk in New Zealand. The original version of the QMRA was developed to assist setting New Zealand Raw Milk Regulations and Guidelines by the MPI (2016). The new development is a part of a 2019 regulatory review, and focusses on the impacts of both farm practices and consumer behaviours.

**Methodology:** In this talk we present a Monte Carlo modelling approach to estimate the risk of illness due to the pathogens VTEC/STEC, *Salmonella* spp. and *Listeria monocytogenes* which are known to grow and/or survive in raw milk. Focusing on the effect of consumer behaviours, whilst assuming farms adhere to MPI regulations, we define consumer refrigerator temperatures as either a single value aligned with MPI advice or a distribution derived from a survey of New Zealand household refrigerators. The risk of illness is then compared under these scenarios for single and daily consumption, taken before the proposed use by date.

**Results:** The results of this QMRA suggest that *Listeria monocytogenes* posed a much lower risk in comparison to STEC and *Salmonella* spp. The estimated number of cases of STEC and *Salmonella* spp. increases as the fridge temperature and/or use by time increases. When considering the risk from a single serving, the estimated number of cases of illness approximately doubles when a use by date is based on 7 days from milking compared to the regulatory 4 days from milking. Similarly, the risk of illness from consuming raw milk refrigerated at 7°C is 3-5 times greater than that refrigerated at the recommended 4°C.

**Conclusion and Relevance:** This QMRA highlights the importance of considering multiple variables, e.g. fridge temperature, use-by-date, and initial contamination, when estimating the risk to public health of drinking raw milk. Further challenges to better quantifying the population health risk include estimating the size of the population at risk and the distribution of raw milk serving size consumed.

**Keywords:** Raw milk; Monte-Carlo simulation; public health; QMRA; regulations

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## 85: A QUANTITATIVE EXPOSURE ASSESSMENT MODEL FOR *LISTERIA MONOCYTOGENES* IN SLICED FERMENTED SAUSAGES DURING PRODUCTION AND DISTRIBUTION CHAIN

Arícia Possas<sup>1</sup>, Vasilis Valdramidis<sup>2</sup>, Rosa María García-Gimeno<sup>1</sup>, Fernando Pérez-Rodríguez<sup>1</sup>

<sup>1</sup>University of Córdoba, Córdoba, Spain. <sup>2</sup>University of Malta, Msida, Malta

**Introduction:** The control and eradication of *Listeria monocytogenes* in the meat industry is a challenge for food managers, due to its psychrotrophic and ubiquitous nature. Food safety criteria concerning *L. monocytogenes* presence in ready-to-eat (RTE) foods, including shelf-stable dry-fermented meat products, vary among countries, but a zero tolerance is often adopted. The development of quantitative tools to predict *L. monocytogenes* levels in these products is therefore relevant to assure compliance with legal requirements.

**Methodology:** A quantitative microbiological exposure assessment (QMEA) model of *L. monocytogenes* in sliced vacuum-packed chorizo sausage was developed. The model comprises the entire manufacturing production and distribution processes, from raw-materials up to consumption, considering the possibility of cross-contamination occurrence during post-process operations and including high-pressure processing (HHP) application as a control measure. Each model step was built based on literature data, predictive models and expert opinions. Monte-Carlo analysis was performed under various scenarios of formulation, processing and storage conditions, providing estimates of bacterial concentration and prevalence at each step.

**Results:** Model simulations indicate that *L. monocytogenes* can survive the manufacturing production process and distribution chain of the sliced vacuum-packed products. Pathogen levels would exceed 100 cfu/g by the end of their shelf-life in case the initial level of pork meat contamination entering the production process was higher than 3.5 log cfu/g. Storage temperatures during the distribution chain steps affect the levels of exposure to *L. monocytogenes* at the consumption phase. Pressure-treatments at 600 MPa/3 min could assure products' compliance with the regulation EC 2073/2005, while treatments at 600 MPa/10 min would be enough to reduce the prevalence of *L. monocytogenes* to 0 % of contaminated packs, considering the worst-case scenario of cross-contamination during post-process operations.

**Conclusion and Relevance:** The QMEA model developed could be successfully applied to assess and validate suitable control measures, *i.e.*, reformulation and HHP application, to assure compliance with the current regulations concerning *L. monocytogenes* presence in sliced vacuum-packed chorizo. Quantitative tools such the QMEA developed in this work are relevant for risk managers to evaluate different preventive strategies to guarantee the compliance with *L. monocytogenes* microbiological criteria for dry-fermented sausages.

**Keywords:** Microbial risk assessment; probabilistic model; Monte Carlo simulation; dry-cured meats; predictive microbiology



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## 106: PROBABILISTIC RISK MODEL OF NOROVIRUS TRANSMISSION DURING HANDLING AND PREPARATION OF FRESH PRODUCE IN SCHOOL FOODSERVICE OPERATIONS

Araceli Bolívar<sup>1</sup>, Junehee Kwon<sup>2</sup>, Kevin Sauer<sup>2</sup>, Dojin Ryu<sup>3</sup>, Ewen Todd<sup>4</sup>, Fernando Pérez-Rodríguez<sup>1</sup>

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**Introduction:** Human noroviruses (NoV) are recognised worldwide as important pathogens and the primary cause of food-borne disease outbreaks from contaminated food in the U.S. They are often transmitted by infected food handlers manipulating foods during preparation, such as fresh fruits and vegetables. Children comprise a portion of the highly susceptible population to NoV disease. This paper provides a study to model the transfer of NoV between food handlers and vegetables during salad preparation in school food services based on direct observation data.

**Methodology:** Three transfer pathways were modelled by considering different initial contamination sources: (1) environment, (2) handlers and (3) contaminated produce. A scenario analysis was performed representing different levels of NoV in the initial contamination sources. The output was expressed in NoV infective particles (NoVP) per serving of produce. The probability of infection was estimated based on the NoV levels at consumption obtained from each simulated transfer pathway using the beat-binomial dose-response model with  $\alpha=0.04$  and  $\beta=0.055$ . A sensitivity analysis was applied to identify the most important model inputs.

**Results:** Pathways 1 and 2 indicated that initial levels of  $\leq 10^4$  NoVP/fomite resulted in  $<0.5\%$  cases per serving of NoV infection. When initial levels were higher, % cases of NoV infection was estimated to be ca. 3%. This rise in % cases of infection was linked to higher doses (5% serving with  $\geq 15$  NoVP/serving) and prevalence levels at consumption ( $>0.2$ ). In Pathway 3, all scenarios could lead to infected individuals, although number of cases of infection was lower ( $<1.3\%$ ), despite concentration levels were higher. The sensitivity analysis indicated that increasing hand washing frequency from 0.2 to 0.8 units resulted in reducing the number of NoV infections from 7% to 1%.

**Conclusion and Relevance:** Based on the sensitivity analysis, NoV transfers to fresh produce may be minimised through effective training programs specifically addressing hand washing. The model in this study, which was incorporated in the risk assessment module of the predictive software tool MicroHibro, might be used to evaluate the impact on the risk associated with NoV transmission of specific and effective training programs, aimed at food chain operators.

**Keywords:** Enteric viruses; microbial risk assessment; cross-contamination; stochastic modelling; lettuce

## Oral Session 8: Predictive Modelling in Food Quality and Safety

Time: 17:00 - 18:10

Date: 19th September 2019

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### 101: MULTI-CRITERIA ANALYSIS APPROACH CONSIDERING FOOD SAFETY, FOOD WASTE AND ENERGY CONSUMPTION: APPLICATION TO PRODUCTION PROCESS OF PUFF PASTRY

Steven Duret<sup>1</sup>, Laurent Guillier<sup>2</sup>, Erwann Hamon<sup>3</sup>, Hong-Minh Hoang<sup>1</sup>, Evelyne Derens-Bertheau<sup>1</sup>, Jean-Christophe Augustin<sup>2,4</sup>, Anthony Delahaye<sup>1</sup>, Onrawee Laguerre<sup>1</sup>, Valérie Stahl<sup>3</sup>

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**Introduction:** The preservation of perishable food *via* cold supply chain is essential to extend shelf life and ensure food safety. However, the use of refrigeration processes requires energy consumption with economic and environmental impact. Increasing the temperature set point in cold equipment could save energy, but the global cost may increase due to food waste or safety issues. The study of potentially conflicting objectives such as food safety, food waste, and energy consumption may be challenging because the comparison between the outcomes of those objectives (risk of illness, amount of food waste, energy consumption in kWh). Methodologies leading to overcome issues involving conflicting impacts and the knowledge of their limitations are essential to support decision makers.

**Methodology:** This study focuses on the modelling of the energy consumption of cold facilities, food temperature, food contamination and growth of *Listeria monocytogenes* and lactic acid bacteria in a puff pastry plant. An event based framework was used to model the main components of the production line (kneader, conveyor, cooling spiral and cold room). Own-checks (microbial and temperature) data from food industry premises were used to inform and parametrize the model. Different scenarios were tested to evaluate the impact of operating conditions (setting temperature and duration) on energy consumption, food waste and food safety.

**Results:** Results showed that the temperature setting of the cooling spiral had a low impact on energy consumption and a high impact on the microbial quality. The opposite phenomena were observed in the cold room with a moderate impact of the cold room temperature setting on the food microbial quality and a high impact on energy consumption. Finally, a multi-criteria decision analysis approach is proposed to identify the optimal operating conditions regarding the three criteria. Criteria weights were set by the operators of the food plant (quality manager, production manager, energy advisor and plant director).

**Conclusion and Relevance:** This methodology helps manufacturers in decision making to optimise operating conditions and reduce energy consumption with a limited impact on food waste and safety.

Keywords: Multi-criteria analysis; food waste; energy consumption; temperature; food plant

## 12: A GENERIC MODEL SYSTEM FOR COLLECTING DATA TO PREDICT MICROBIAL SPOILAGE OF COMMERCIALLY PACKAGED FRESH-CUT VEGETABLE SALADS USING TEMPERATURE AND IN-PACKAGE CO<sub>2</sub> LEVELS AS PREDICTORS VARIABLES

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**Introduction:** Modified atmosphere packaging and storage temperature are two hurdles capable of slowing down changes of gases inside the packaging and consequently extending the shelf-life of fresh-cut salads. Existing predictive models are commonly constrained to the product and packaging conditions for which they were developed. The aim of the present study was to develop a vegetable-packaging model system in order to minimise the effect of packaging characteristics and post-harvest physiology, i.e., film permeability, headspace volume, and respiration rate; and predict only the impact of temperature and in-package gas composition on microbial spoilage of fresh produce.

**Methodology:** Ten g of rocket pulp (model food) were packaged (low permeability film) in different O<sub>2</sub>/CO<sub>2</sub> ratio atmospheres (0 to 20% O<sub>2</sub>, 20 to 0% CO<sub>2</sub> with N<sub>2</sub> at 80%) and stored at 0-15°C. Gas composition was monitored *via* gas meter. Pseudomonads and lactic acid bacteria (LAB) growth was primary modelled using Baranyi model, while a polynomial model was used for modelling  $\mu_{max}$  as a function of temperature and CO<sub>2</sub>.

**Results:** Growth models were validated under various commercial fresh-cut vegetables of high respiration rate, MAPs, packaging films, and at isothermal/dynamic temperature conditions. Variations in the package gas concentration of rocket pulp was either eliminated or significantly decreased ( $p < 0.05$ ), under different MAP conditions, thus adequately verifying the initial hypothesis of the developed vegetable/packaging model system. Pseudomonads and LAB were recorded as the most important microbial groups (dominant and of high growth dynamics, respectively). Both polynomial models had acceptable performance, since they met the criterion of pRE (proportion of relative error)  $\geq 0.70$ , along with presenting bias factor of 0.99 (LAB) or 1.00 (pseudomonads) and accuracy factor of 1.07 (LAB) or 1.04 (pseudomonads). The developed polynomial models for  $\ln(\mu_{max})$  of LAB and pseudomonads showed  $R^2_{adj}$  of 0.975 and 0.839, respectively.

**Conclusion and Relevance:** The proposed vegetable-packaging model system is a practical and easily implemented approach that may assist the fresh produce sector in

predicting the shelf-life of a group of fresh-cut salads i.e., high respiration rate, under different packaging and storage conditions.

Keywords: Model food; fresh-cut salads; CO<sub>2</sub>; temperature; predictive modelling

### **32: LATENT DIRICHLET ALLOCATION IN FOOD SPOILAGE ANALYSIS: A CASE STUDY WITH ATLANTIC SALMON (*SALMO SALAR*)**

Lotta Kuuliala<sup>1,2</sup>, Raúl Pérez-Fernández<sup>2</sup>, Mengzi Tang<sup>2</sup>, Bernard De Baets<sup>2</sup>, Frank Devlieghere<sup>1</sup>

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<sup>2</sup>Department of Data Analysis and Mathematical Modelling / Ghent University, Ghent, Belgium

**Introduction:** Recent advances in metabolomics and real-time mass spectrometry have led to an increasing interest towards multivariate statistical analysis and mathematical modelling in volatile-based food quality research. However, the complexity of spoilage processes poses a major challenge in identifying potential spoilage indicators and in developing associated models. The aim of this study was thus to use probabilistic topic modelling, more specifically Latent Dirichlet Allocation (LDA), for examining the quality deterioration of Atlantic salmon (*Salmo salar*).

**Methodology:** Previously collected datasets (Kuuliala *et al.* 2019, Int J Food Microbiol 303:46-57) regarding the microbiological, chemical and sensory quality of salmon under different gaseous atmospheres (4 °C) were used in the study. LDA topics were generated by examining the distribution of 25 volatile organic compounds (VOCs) within individual salmon samples from different stages of storage. Firstly, exploratory analysis was carried out for 1) determining the identification criteria of salmon quality status and 2) optimizing the model performance. Subsequently, selective analysis was performed for identifying the most important volatile spoilage indicators under different storage conditions.

**Results:** On the basis of VOC distributions within topics and the correlation of topic distributions with microbiological and sensory quality, the extracted topics (VOC profiles) could be interpreted and related with different salmon quality stages. Consequently, critical quality thresholds based on changes in topic distributions throughout storage time could be determined. The identified spoilage indicators were well in accordance with results obtained with Partial Least Squares (PLS) regression.

**Conclusion and Relevance:** Overall, LDA was found to provide a versatile view into salmon quality deterioration and to possess several advantages over basic multivariate methods, such as PLS. Irrespective of the analytical technique used for characterizing and quantifying the food volatile, the extraction of information from the resulting datasets typically calls for advanced statistical analysis. However, overcoming the limitations of basic multivariate methods requires more flexible approaches, particularly at the exploration stage. The outcomes of the present study can be considered not only to facilitate food spoilage characterization, but also to advance packaging technology development.

Keywords: Mathematical modelling; metabolomics; multivariate statistical analysis; real-time mass spectrometry; volatile organic compound

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#### **44: PRACTICAL APPLICATION OF PREDICTIVE MODELS FOR MEAT PRODUCTS - CHALLENGES AND OPPORTUNITIES**

Antonia Albrecht<sup>1</sup>, Martin Hebel<sup>1,2</sup>, Ulrike Herbert<sup>2</sup>, Claudia Waldhans<sup>1</sup>, Imke Korte<sup>1</sup>, Sophia Dohlen<sup>1</sup>, Judith Kreyenschmidt<sup>3</sup>

<sup>1</sup>Institute of Animal Science, University of Bonn, Bonn, Germany. <sup>2</sup>Dr. Berns Laboratorium GmbH & Co. KG, Neukirchen-Vluyn, Germany. <sup>3</sup>Department of Fresh Produce Logistics, Geisenheim University, Geisenheim, Germany

**Introduction:** Predictive modelling is a tool of vital importance for the assessment of shelf life and prediction of the product status under real chain conditions. With appropriate temperature monitoring solutions the current status of the product can be calculated at every step of the chain, offering the opportunity to introduce a dynamic shelf life. But the implementation of microbial models under practical conditions is hindered by several challenges. Major tasks are the estimation of the initial quality of fresh meat from industrial production as well as the high variability of microbial data.

**Methodology:** For investigating the impact of these traits on the reliability of predictive models, the initial contamination of over 800 samples of fresh pork, poultry, lamb and beef was analyzed. A meta-analysis covering various packaging (aerobe, map, vacuum) and products was conducted on over 500 storage trials to explore methodical complexities during the assessment of shelf life under different conditions. Total viable count (TVC) and typical spoilage organisms were determined by classical enumeration methods. The deterioration of the samples was investigated in storage trials with subsequent microbial and sensory evaluations to characterize the spoilage process.

**Results:** Different primary and secondary level models were applied to model shelf life and to explore the ability to predict the real product status. For investigating data variability and uncertainty of models, error estimates and probabilistic approaches were applied. Production and storage characteristics such as seasonality, hygiene, product composition and packaging showed a significant influence on microbial variability and model accuracy. Based on these factors, the prediction of variability was used to improve the accuracy of shelf life models.

**Conclusion and Relevance:** These improvements for the practical application of predictive models enhance cold chain management, optimise product handling and support the prevention of food waste along the chain. Reliable predictive modelling improves the overall life cycle assessment of new packing materials by calculating the effect of the packaging on shelf life and potential to prevent food waste. This allows for a more realistic calculation of the environmental impact of innovative packaging solutions for meat products.

Keywords: Data variability; model applicability; spoilage models; fresh meat

## 86: PREDICTING THE EFFECT OF CARVACROL ON THE EFFLUX PUMP ACTIVITY IN *ESCHERICHIA COLI*

Anna Jánosity<sup>1</sup>, Anja Klančnik<sup>2</sup>, Gabriella Kiskó<sup>1</sup>, Sonja Smole Možina<sup>2</sup>, József Baranyi<sup>3</sup>

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**Introduction:** The food industry routinely uses antibiotics, even in healthy animals, to promote growth and prevent infectious diseases. This practice contributes to the spread of multidrug-resistant (MDR) microorganisms and has a direct effect on the microbiological safety of various foods. In this study, we use predictive microbiology methods to describe the efflux-pump-modulating activity of carvacrol, a substance, naturally present in essential oils and commonly used for human food consumption via herbs.

**Methodology:** We used *Escherichia coli* strains as model microorganisms. Ethidium bromide (EtBr) played the role of a surrogate for antibiotics. Its spread in the cell was measured by Varioskan LUX multimode microplate reader (Thermo Scientific) with real-time fluorescent ethidium bromide efflux assay during a 1-hour-long experiment, repeated three times with three replicates for each. Since the rate, at which the EtBr spread in the cell, was the fastest in the first quarter of the experimental time, a saturation function was fitted to the “EtBr v. time” data. We considered its maximum rate,  $r$ , estimated by the saturation model, as the primary model parameter. The  $c$  proportion of the carvacrol concentration to the MIC (minimum inhibitory concentration), was chosen as the explanatory variable for secondary modelling, where MIC was experimentally determined as 300 mg/L. In our experimental design, the test interval for  $c$  was 0.1 - 0.5. The efficiency of the carvacrol was quantified by the  $Dr/Dc$  ratio, which was described by a bi-phasic secondary model.

**Results:** The efficiency of the carvacrol increased with its concentration from a threshold point which was found to be between 0.2 and 0.3 MIC. We also show that carvacrol was generally more effective than another standard EPI (NMP – 1-(1-Naphthylmethyl)-piperazine) that we tested.

**Conclusion and Relevance:** Predictive microbiology methodology proved to be a suitable tool to show that carvacrol is an effective EPI. Our final predictive model can be used to optimise the application of carvacrol as a natural surrogate for EPI-s. Standard EPI-s are generally not permitted antimicrobials because of their potential toxicity. Carvacrol, with its resistance-weakening activity, it presents a new strategy to prevent the spread of MDR bacteria.

**Keywords:** Efflux pump inhibitors; carvacrol; antibiotic resistance; inactivation kinetics; *Escherichia coli*

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## 15: STOCHASTIC EVALUATION FOR SURVIVAL BACTERIAL NUMBERS AND THE TIME-TO-INACTIVATION BY USING WEIBULL MODELLING AND MONTE CARLO SIMULATION

Satoko Hiura, Kento Koyama, Hiroki Abe, Shige Koseki

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**Introduction:** Enterohemorrhagic *Escherichia coli* and *Salmonella enterica* can cause foodborne illness even if they were ingested as less than 100 cells. Previous studies have shown that the variability in inactivation behaviour remarkably increases in less than 100 cells. Thus, bacterial behaviours with small numbers cannot be properly assessed by conventional deterministic kinetic models. In recent years, stochastic models that describe variability in the number of bacteria and individual cell heterogeneity are attracting attention. In this study, we aimed to clarify the inactivation behavior of *E. coli* under low pH environment and to develop a predictive model for survival probability by using Weibull modelling and Monte Carlo simulation.

**Methodology:** We conducted acidified inactivation of *E. coli* at pH 3.0 with 3 replications of  $10^5$  initial cells for model development and 50 replications of  $10^1$ ,  $10^2$ , and  $10^3$  initial cells for model validation. Monte Carlo simulation was carried out using parameters obtained by Weibull fitting for triplicate survival kinetic data from  $10^5$  cells. We assumed that inactivation of individual cell is independent and initial cell number follows Poisson distribution. Weibull distribution describes inactivation timing of each cell as independent event. We generated random numbers as many as the initial cell numbers derived from the Weibull distribution to obtain the time that each one bacterial cell requires to be inactivated ( $= t_i$ ). At an arbitrary time ( $t$ ), if  $t < t_i$ , the bacterial cells were judged as survival, otherwise if  $t > t_i$ , the bacterial cells were judged to be inactivated.

**Results:** The survival kinetics of  $10^5$  initial cells was accurately described by Weibull model. The obtained Weibullian parameters enabled to successfully predict survival kinetics in the other initial cell numbers such as  $10^1$ ,  $10^2$ , and  $10^3$ . Monte Carlo simulation by using the obtained Weibullian parameters successfully predicted the variability in the survival ratio and the time-to-death as a probability distribution regardless of initial cell numbers.

**Conclusion and Relevance:** The developed Monte Carlo simulation procedure enabled to predict variability in inactivation behaviour at various contamination level by using a conventional deterministic model parameters from a large initial cell numbers.

**Keywords:** Monte Carlo simulation; variability; probability; Weibull distribution; Poisson distribution

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## Oral Session 9: Predictive Mycology

Time: 9:40 - 10:40

Date: 20th September 2019

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### 95: MODELLING THE INACTIVATION OF *NEOSARTORYA FISCHERI* ASCOSPORES IN CLARIFIED APPLE JUICE BY DIFFERENT ULTRAVIOLET RADIATION INTENSITY

Natielle Maria Costa Menezes, Beatriz Oliveira Ortiz, Charles Kautzmann, Agenor Furigo Junior, Gláucia Maria Falcão Aragão

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**Introduction:** *Neosartorya fischeri* ascospores are often associated with the pasteurised fruit juices. Non-thermal technology such as UV-C light could be alternative to reduced heat treatment temperatures. The main objective was to study the effect of UV-C treatments, with different intensities, on *N. fischeri* ascospores inactivation in apple juice.

**Methodology:** *N. fischeri* was isolated and identified from an apple nectar processing line. Apple juice samples (30 mL, pH 3.6, 12  $\pm$ 0.1 °Brix) were inoculated with 0.1 mL of ascospores suspension (4 weeks) ( $10^6$  CFU/mL). UV-C treatments were carried out in a camera (75x70x45 cm<sup>3</sup>) with germicidal lamps (peak emission at 254 nm) and continuously stirred at the intensities of 6.5, 13, 21 and 36 W/m<sup>2</sup> and times from 0 to 30 min (previous lab tests). Log linear plus tail model was fitted to obtained survival curves and goodness of fit of this model was investigated. Logarithmic model secondary was assessed to describe the influence of the intensity of UV-C light on the inactivation parameters.

**Results:** Log linear plus tail model resulted in a good performance statistical indexes (0.300-0.4386 RMSE and 0.96-0.98  $R^2$ ). At high intensity levels tested (36 and 21 W/m<sup>2</sup>) the UV-C light allowed reducing 5 log-cycles in approximately 8 min. When the low intensities were applied, 13 and 6.5 W/m<sup>2</sup>, the time required was 16 and 30 min to attain a similar reduction, respectively. The  $k_{max}$  parameter (specific inactivation rate) increased from 0.08 at 6.5 W/m<sup>2</sup> to 0.32 min<sup>-1</sup> at 36 W/m<sup>2</sup>, demonstrating this parameter is dependent within the intensity of UV-C. UV-C radiation was faster than thermal treatment at 90 °C (literature) for obtained the same microbial reduction. For  $N_{res}$  parameter, the secondary model was considered the mean values ( $N_{res}=-5.56$ ). The logarithmic model ( $k_{max}=0.147*\ln(x)-0.19$ ;  $R^2=0.92$ ) was a good model to describe the influence of UV-C light intensity on  $k_{max}$  parameter.

**Conclusion and Relevance:** Accordingly, experimental results obtained model can predict *N. fischeri* ascospore inactivation, in the assessed intensities range. UV-C light technology is a promising application for preventing the spoilage of juices and reduce of nutritional impacts.



Keywords: Ultraviolet light (UV-C); predictive mycology; apple juice; non-thermal technologies

#### 49: A BIGELOW-TYPE META-REGRESSION MODEL DESCRIBING THE HEAT RESISTANCE OF *TALAROMYCES* SPORES

Veronica Alvarenga<sup>1,2</sup>, Leonardo Prado-Silva<sup>2</sup>, Vasco Cadavez<sup>3</sup>, Ursula Gonzales-Barron<sup>3</sup>, Anderson Sant'Ana<sup>2</sup>

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**Introduction:** Heat resistant fungi (HMR) belonging to genera such as *Byssoschlamys*, *Talaromyces*, *Neosartorya* and *Eupenicillium* are frequently isolated from thermally processed fruit products. Because of the great challenge they pose to the fruit juice industry, several researchers have estimated the inactivation parameters of HMR aiming to further design proper heat processing regimes that ensure shelf-stable products.

**Methodology:** The study developed a meta-analytical model to describe the thermal inactivation of *Talaromyces* spp. in fruit juices, using data extracted from 6 studies, published between 1969 and 2017. A Bigelow-based equation of D values as a function of temperature, pH and °Brix, moderated by inactivation method and presence of preservatives, was fitted to 196 data points as a weighted mixed-effects linear regression. Overall D\* (D at T=90°C, pH=3.5 and °Brix=12), z<sub>T</sub>, z<sub>pH</sub> and z<sub>Brix</sub> values were estimated.

**Results:** The D value of *Talaromyces* spp in fruit juices was affected by temperature (p<0.001), °Brix (p<0.001), pH (p=0.030), use of preservatives (p<0.001) and inactivation method (p<0.001). The highest overall D value were found for screwcap tubes (SCW) (D\* = 5.25 min; 95% CI: 4.25-6.50) and the lowest value was for polyethylene bags (D\* = 1.66 min; 95% CI: 1.13-2.42). The overall D\* of juices using SCW were 4.58 min, 4.91 min, 6.58 min and 5.18 min, for apple, orange, pineapple and strawberry, respectively. When TDT was used as the inactivation method, lower D\* were estimated (apple: 2.19 min; orange: 2.35 min; pineapple: 3.15 min and strawberry: 2.48 min). The overall z<sub>T</sub> of *Talaromyces* was estimated at 6.86°C (95% CI: 6.38-7.35°C); yet, when adding preservatives, z<sub>T</sub> value increased to 10.09°C (95% CI: 8.83-11.35°C). The overall z<sub>pH</sub> value for *Talaromyces* was estimated at 7.05 (95% CI: 5.33-12.68) while for the first time an overall z<sub>Brix</sub> value was estimated (85.32; 95% CI: 65.28-105.4) to characterise *Talaromyces*.

**Conclusion and Relevance:** The overarching Bigelow-type model developed allows the estimation of thermal death parameters of *Talaromyces* spp. ascospores in juices from temperature, soluble solids contents, pH, use of preservatives and fruit. Moreover, the findings underscore that the choice of the inactivation method is critical in the quantification of D\* and z values.

Keywords: Meta-analysis; heat resistant fungi; thermal inactivation; fruit juice; heat processing

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## 77: EFFECT OF 70% ETHANOL SOLUTION ON THE INACTIVATION OF FOUR STRAINS OF *PENICILLIUM BIFORME*: IMPACT OF THE PHYSIOLOGICAL STATE OF THE CONIDIA

Vincent Visconti, Karim Rigalma, Emmanuel Coton, Philippe Dantigny

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**Introduction:** *Penicillium biforme* (formerly *Penicillium commune*) is one of the main fungal contaminants in the dairy industry. In this context, 70% ethanol is widely used as a surface disinfectant. Little information on ethanol efficiency against *P. biforme* is available. According to the international standards, disinfectants efficiency against fungi is assessed using fungal conidia obtained from specific species mycelium grown at optimum conditions (0.995, water activity,  $a_w$ ) and re-suspended into physiological water. These conditions did not reflect the physiological state of conidia produced in an industrial environment. The objective of the present study was to assess the effect of the physiological state on the inactivation of four *P. biforme* strains due to ethanol.

**Methodology:** *P. biforme* mycelia were grown on PDA (Potato Dextrose Agar) medium at either 0.995 or 0.950  $a_w$ . After 7 days incubation at 25 °C, conidia were either dry-harvested or suspended in physiological water for 30 min. Then, 70% (vol/vol) ethanol solution was used for 1, 2 and 5 min. The initial conidia quantity,  $N_0$ , was evaluated by microscopy on a haemocytometer whereas the post-treatment survivors,  $N$ , were assessed through their ability to germinate and to form mycelium after 7 days incubation at 25 °C.

**Results:** At 1 min treatment, survivors were obtained only for dry-harvested conidia produced at 0.95  $a_w$  for all the strains. For these conditions, about 5 log reductions were obtained for 3 strains whereas the most resistant UBOCC-A-116003 strain exhibited a 2.35 log reduction only. At 2 min treatment, dry-harvested conidia of the most resistant strain produced at 0.95  $a_w$  exhibited survivors. Whatever the strain and the physiological state of the conidia, no survivors were observed for 5 min treatments.

**Conclusion and Relevance:** More than 5 log reductions were achieved for 5 min application of 70% ethanol. Shorter application times should be avoided in the dairy industry. The physiological state of conidia as well as the strain should be taken into account when assessing the efficacy of disinfectants.

**Keywords:** *Penicillium biforme*; ethanol; water activity; dry-harvesting; intraspecific variability

### 13: MODELLING OF THE EFFECT OF GRAIN STEEPING DEGREE, AND WHEAT GERMINATION TEMPERATURE AND TIME ON AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS* DURING WHEAT MALTING FOR CRAFT BEER

Danieli Cristina Schabo<sup>1,2</sup>, Marta Hiromi Taniwaki<sup>3</sup>, Donald Schaffner<sup>4</sup>, Marciane Magnani<sup>2</sup>

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**Introduction:** *Aspergillus flavus* is one of the most widely distributed potentially toxigenic fungal species in nature. Grains of wheat used to produce malt from craft beer infected by *A. flavus* in the field or during storage and malting may favor the production of aflatoxin (AF). The objective of this study was to develop a mathematical model describing the effect of three factors (steeping degree, temperature and wheat germination time) on AFB<sub>1</sub> and AFB<sub>2</sub> production during malting of wheat grains.

**Methodology:** Wheat grains were inoculated with a spore suspension (10<sup>6</sup> spores/mL) of an AFB<sub>1</sub> and AFB<sub>2</sub> producer *A. flavus* strain. The malting of wheat grains was performed under varied steeping degree (35, 42, 43 and 45%), germination temperature (13, 15, 17 and 19 °C) and germination time (48, 72, 96 and 120 h) in a full factorial design of 64 experiments. The germinated grains were kilned and the rootlets manually removed. Samples (~50 g) were taken in duplicate and AFB<sub>1</sub> and AFB<sub>2</sub> were determined by HPLC. A multiple linear regression was used to estimate mycotoxin-production correlated with steeping degree and temperature and time of wheat germination. The model was built using R software.

**Results:** Under all assayed conditions, levels of AFB<sub>1</sub> and AFB<sub>2</sub> produced exceeded the maximum levels established for cereals and derived products by the European Commission (2 µg/kg for AFB<sub>1</sub> and 4 µg/kg for total AF) and limits determined by the Food and Drug Administration in foods for human consumption (20 µg/kg for total AF). A simple linear regression model revealed that there was a negative but non-significant relationship between AFB<sub>1</sub> and AFB<sub>2</sub> production and the steeping degree of wheat grains. Wheat germination temperature and germination time were significantly ( $p < 0.001$ ) negatively and positively correlated with aflatoxin production, respectively. Production of AFB<sub>1</sub> and AFB<sub>2</sub> decreases with increase of germination temperature, while increases with increase of germination time of wheat grains.

**Conclusion and Relevance:** Current conditions proposed for wheat malting allow the AFs in grains by toxigenic strains. The regression equation can serve as a predictive tool to reduce, but not prevent the AFB<sub>1</sub> and AFB<sub>2</sub> in wheat malt for craft beer.

**Keywords:** Predictive mycology; aflatoxins; malting process; wheat malt

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## 84: RISK ASSESSMENT MODELLING OF PASTEURISED FRUIT PUREES SPOILAGE BY HEAT RESISTANT MOULDS (HRM)

Juliana L. P. Santos<sup>1</sup>, Jeanne-Marie Membre<sup>2</sup>, Simbarashe Samapundo<sup>1</sup>, Liesbeth Jacxsens<sup>1</sup>, Jan Van Impe<sup>3</sup>, Anderson S. Sant'Ana<sup>4</sup>, Frank Devlieghere<sup>1</sup>

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**Introduction:** Heat resistant moulds (HRMs) are well known as specific spoilage organisms (SSOs) of pasteurized fruit products. Thus, this study aimed to develop a quantitative approach to determine the spoilage risk due to HRMs in packaged fruit purees at the time of use under various scenarios regarding formulation, processing and storage.

**Methodology:** The study was focused on three types of fruit purees considering original data collected for *Neosartorya fischeri* and *Byssoschlamys nivea*. The development of the risk assessment comprised three steps: (1) distribution of initial contamination level of raw material by ascospores ( $N_0$ ), (2) inactivation of ascospores during thermal processing ( $N_p$ ) and (3) determination of the number of ascospores which are able to survive thermal processing and present visible mycelia ( $D=2\text{mm}$ ) during storage ( $N_f$ ). Data of visible growth comprised distributions obtained as function of  $a_w$ -values (0.86-0.98), oxygen (0-21%), temperature (8-30°C) and pasteurization (95-105°C/15sec). Each 'risky' pack represented a 100 g pack of fruit puree contaminated with at least one ascospore ( $N_f$ ) able to present visible growth within the typical use or consume by dates. The model was developed in R version 3.5.2. Uncertainties were taken into account in the risk prediction. The simulations performed consisting of  $10^5$  iterations (= number of packs).

**Results:** It was estimated that 66% of strawberry puree packs ( $a_w = 0.890$ ) which received a mild, but commonly applied heat treatment i.e. 85°C for 15sec to 1min, were spoiled within two months when the packs were stored at 30°C. Reduction of the spoilage risk by 90% was predicted to occur when the pasteurization intensity was increased i.e. to 95°C (15 sec-1 min), and by 40% by decreasing the  $a_w$ -value to 0.870 and storing the puree at 22°C.

**Conclusion and Relevance:** Through this study, it was possible to gather various types of data on HRMs and translate them into the risk (= number of spoiled products) under various scenarios. The results can be used to support risk management decisions in identifying and quantifying the impact of possible interventions during formulation, processing and storage of fruit purees to eliminate or greatly reduce this risk.

Keywords: Ascospores; storage; spoilage risk; fruit puree; shelf-life

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## Oral Session 10: Individual Cell and Whole Cell Modelling

Time: 11:00 - 11:50

Date: 20th September 2019

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### 14: OPTIMISED EXPERIMENTAL DESIGN TO STUDY THE PROBABILITY OF GROWTH OF INDIVIDUAL BACTERIAL CELLS

Nathália Buss da Silva<sup>1</sup>, Bruno Augusto Mattar Carciofi<sup>1</sup>, Mariem Ellouze<sup>2</sup>, József Baranyi<sup>3</sup>

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**Introduction:** This work proposes an improved experimental design to estimate the probability that a single cell, in a homogeneous bacterial population, produces a growing subpopulation. Predicting this probability of growth is especially important when a low number of pathogenic cells can compromise the microbial safety of foods.

**Methodology:** Our method is based on a comparison between an *a-priori* (before inoculation) and an *a-posteriori* (after detection of growth) estimator of the number of cells in a well of a micro-titre plate. The former is based on plate counts, the latter is based on the fraction of inoculated but non-turbid wells. Here, we analyse both the efficiency and accuracy of the *a-posteriori* estimator and modify it to be used when random low number of cells are inoculated into the wells.

**Results:** We demonstrate that an optimal scenario is when ca. 20% of the wells do not produce growth. In this case, (i) the new estimator is close-to-unbiased, and (ii) the relative error of the estimate has a local minimum, making the probability of growth estimation more reliable. Based on this, recommendations on the targeted number of cells per well, ranging from 0.9 to 3.0 cells/well, can be drawn.

**Conclusion and Relevance:** When more than 200 wells are studied, the *a-posteriori* estimator has 10% or less relative deviation. This is affordable, from an experimental point of view, since one can use a simple incubator and observe growth/no growth of the targeted organism in 200 wells. In that case, the method offers a way to measure the probability of growth with one digit accuracy. However, it is not feasible to identify changes in the probability of growth when it is close to 100%, and only differences in this probability greater than 10% are detectable. Relatively small changes in the environmental conditions can induce detectable changes in the probability of growth for single cells. We presented a means to optimise experimental designs when assessing this parameter.

**Keywords:** Probability of growth; low inoculum; experimental design

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## 19: DEVELOPMENT AND VALIDATION OF CALCULATION FRAMEWORK FOR STOCHASTIC PREDICTION OF SURVIVAL SPORE BEHAVIOUR DURING ISOTHERMAL AND NON-ISOTHERMAL INACTIVATION PROCESS WITH SECOND-ORDER MONTE-CARLO SIMULATION BASED ON A NON-PARAMETRIC BOOTSTRAP METHOD

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**Introduction:** Kinetic survival models, such as the model based on *D*-value, conventionally support the decisions on thermal inactivation conditions in food processing. Although these models estimate changes in survival cell numbers over time, the variance of the survival counts which are attributed to the variability and uncertainty of kinetic data are not estimated. A stochastic model appropriately describing variance of survival cell counts will enable to optimise risk-based inactivation conditions. This study aimed to develop a stochastic model considering “variability” and “uncertainty”. The model developed estimates the variance of survival behaviour during isothermal and non-isothermal inactivation based on kinetic data. Furthermore, the estimated variances were compared with the variance of observed values.

**Methodology:** A strain of *Bacillus simplex* was used as psychrophilic spore-forming bacteria. The spores were heated under several isothermal conditions (pH: 5.4, 5.8, 6.2, 6.6 and 7.0; Temperature: 80, 85 and 90°C). The changes in the survival spore count were determined by plate counting. The distribution of fitted parameters of the Weibull model and the secondary model were estimated using a non-parametric bootstrap method. The changes in the survival spore behaviour during non-isothermal heating were estimated using second-order Monte Carlo (2D-MC) simulation. The 2D-MC describes the variance of fitting derived from bootstrap (uncertainty), and the true heterogeneity derived from whether one individual spore is dead or alive during infinitesimal heating time assuming a binomial process (variability). In parallel, the variance in survival kinetics of *B. simplex* spore during non-isothermal treatment was examined in order to compare it with the estimated survival counts value using the conventional method of plate counting.

**Results:** In all of the thermal histories, the variances in survival spore counts were successfully described with the developed 2D-MC simulation model. The survival probability of *B. simplex* during heating showed a sigmoidal decrease. In addition, the 2D-MC simulation can also estimate the distribution of survival spore counts or the changes in survival probability of spore populations.

**Conclusion and Relevance:** Second-order Monte Carlo simulation based on the bootstrap is useful to describe the variance of bacterial survival behaviour, including both variability and uncertainty, under not only static but also dynamic thermal treatment.

**Keywords:** *Bacillus simplex*; individual cell heterogeneity; heating; variability; uncertainty

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## 27: MULTIPARAMETRIC CHARACTERISATION OF THE MICROENVIRONMENT OF FOODSTUFFS BY NUCLEAR MAGNETIC RESONANCE (NMR): THE PROS WITHOUT THE CONS OF TARGETED ANALYSES APPLIED FOR PREDICTIVE MICROBIOLOGY PURPOSES

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**Introduction:** Numerous mathematical models have been proposed to describe bacterial population behaviors in foods. These models depict the growth kinetics of particular bacterial strains based on their cardinal values. Recently, these traditional macro-environmental approaches have been complemented for the food-borne pathogen *Listeria monocytogenes* by an individual-based modelling (IBM) focusing only on a few cells and their surrounding microenvironment. It takes into account the single-cell growth probability according to key physicochemical parameters of the food matrix and its storage temperature<sup>1,2</sup>.

**Methodology:** In this context, there is an increasingly prominent issue to accurately characterize the physicochemical properties of foodstuffs, namely pH, water activity ( $a_w$ ), as well as NaCl, organic acid and total phenol concentrations. Usually, all these product features are determined using one destructive analysis per parameter at macroscale (> 5g). Such approach prevents an overall view of these characteristics on a single sample. Besides, it does not take into account the intra-product micro-local variability of these parameters within foods.

**Results:** Nuclear Magnetic Resonance (NMR) is a versatile non-destructive spectroscopic technique that can simultaneously measure the physicochemical parameters of interest without extensive preparation. In this work, we designed a dedicated NMR approach to characterize the microenvironment of foods using a single 10-mg sample. The multi-parametric mesoscopic-scale approach was validated on four food matrices: a smear soft cheese, cooked peeled shrimps, smoked salmon and smoked ham.

**Conclusion and Relevance:** This analytical development and its successful application can help address the shortcomings of monoparametric methods traditionally used for predictive microbiology purposes. It opens new doors for the systematic spatial characterization of foodstuffs at micro-local scale in view of improving and fine-tuning predictive microbiology models. It also paves the way to new practices in food safety management and process optimisation.

### References:

<sup>1</sup>Augustin et al. *Food Microbiology*, 2015, 45, 205-215.

<sup>2</sup>Ferrier et al. *Appl. Env. Micro*, 2013, 79, 5870-5881

**Keywords:** Micro-environment; multi-parametric characterisation; food; nuclear magnetic resonance

## **71: DETERMINATION OF GROWTH PROBABILITY AND LAG TIME AT SINGLE-CELL LEVEL BY THE USE OF AN AUTOMATED MICROSCOPY METHOD**

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**Introduction:** An increased number of studies in quantitative microbiology have shown that lag time and probability of growth is much more uncertain and difficult to predict compared to growth rate. However, the validity of a mathematical model in predicting the conditions that lead to critical levels in foods highly depends on its ability to describe the effect of the environment on lag time and growth probability. This implies that a better understanding of the pathogen behavior at single-cell level near growth limits is of great importance in food safety risk assessment. The classical method for individual cell lag times acquisition is based on turbidity measurement on microplate wells inoculated with approximately one bacterial cell per well. The growth probability can be determined by monitoring visually 96-microwell plates and deduced from concentrations estimated with the MPN calculation. That method is labour intensive and would benefit of a higher throughput. In this study, we developed a quantitative experimental investigation using an automated microscopy method to determine the single cell growth probability and lag time.

**Methodology:** The developed microscopy method based on direct cell observation with a phase-contrast microscopy equipped with a 100× objective and a high-resolution device camera. The method is directly inspired by the method proposed Koutsoumanis and Lianou (2013). But instead of a non-destructive method that measures the growth of few colonies with time lapse, a destructive method based on the observation of high numbers of colonies for several times was used.

**Results:** The results of this newly developed method was compared to indirect-method based on the observation of time to turbidity. The growth probabilities and single-cell lag times of four strains of *Listeria monocytogenes* were acquired at 4°C. The number of values acquired for each experiment was similar for both microscopic and indirect method. The microscopic method permit to shorten the duration of the experiments by a factor of 10 compared to the indirect method.

**Conclusion and Relevance:** The microscopic method was shown to be a promising method for studying the behaviour of single-cells.

**Keywords:** Single cell; probability of growth; lag time; cold conditions; *Listeria monocytogenes*



## POSTER PRESENTATIONS

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### Poster Session 1: Advances in Predictive Microbiology Modelling, and Predictive Modelling in Innovative Food Processing and Preservation Technologies

Time: 10:20 - 10:50

Date: 18th September 2019

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#### 5: MODELLING THE EFFECTS OF TEMPERATURE AND LACTATE ON THE GROWTH OF *BACILLUS CEREUS* IN COOKED RICE

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**Introduction:** Rice-based food products are consumed worldwide and frequently linked to foodborne illnesses caused by *Bacillus cereus*. Its spores can survive cooking temperature and outgrow to produce toxins at temperatures commonly for storage of rice-based products. This study modelled the growth behaviour of *B. cereus* in cooked rice as affected by temperature and sodium lactate, a potential antimicrobial additive for rice-based food products.

**Methodology:** The growth curves of four *B. cereus* (mesophilic, two emetic strains and two diarrheal strains) in cooked rice at several dynamic storage temperatures between 1-48°C and in cooked rice containing 0-3% sodium lactate stored at 10, 16, 22, and 30°C were analysed to develop predictive models.

**Results:** A tertiary growth model was developed from the one-step dynamic analysis and showed that the minimum, optimum, and maximum growth temperatures of *B. cereus* in cooked rice were 8, 38, and 47°C, respectively, with an optimum specific growth rate of 0.96 log CFU/h in cooked rice. The model was validated using additional growth curves not included in the model development, and the root-mean-square-error of prediction was 0.45 log CFU/g, indicating that the model is accurate in predicting the growth of *B. cereus* in cooked rice. In cooked rice containing 1-3% lactate stored at 16, 22, and 30°C, the lag phase durations of *B. cereus* were 10-25, 5-15, and 2-4 h, respectively, and the growth rates were 0.11-0.10, 0.20-0.13, and 0.57-0.30 log CFU/h, respectively, whereas *B. cereus* grew significantly slower at 10°C and its maximum populations were <5.0 log CFU/g at ≤16°C with 3% lactate. The effect of temperature and lactate concentration on

the lag phase and growth rate of *B. cereus* could be described by polynomial models and showed that the increase of lactate concentration significantly extended the lag phase and reduced the growth rate of *B. cereus* at  $\leq 22^{\circ}\text{C}$ .

**Conclusion and Relevance:** These models can be used to predict the growth and survival of *B. cereus* in cooked rice as affected by storage temperature and lactate concentration to assess its risk in cooked rice exposed to a wide range of storage temperature.

Keywords: Modelling; *Bacillus cereus*; rice; temperature; lactate

## 21: EXTENDED CARDINAL PARAMETER GROWTH AND GROWTH BOUNDARY MODEL FOR NON-PROTEOLYTIC *CLOSTRIDIUM BOTULINUM* – EFFECT OF TEN ENVIRONMENTAL FACTORS

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**Introduction:** Growth and toxin formation by non-proteolytic *Clostridium botulinum* in chilled seafood can be managed with  $\geq 3.5\%$  water phase salt (WPS). However, recent dietary recommendations suggest reduced salt intake due to negative health effects of sodium and therefore, other environmental factors should be used to prevent growth of *C. botulinum*. The aim of this study was to expand an available growth and growth boundary model for non-proteolytic *C. botulinum* with terms for  $\text{CO}_2$  and smoke components (phenol) to predict growth responses and facilitate product development as well as documentation of food safety for MAP smoked seafood.

**Methodology:** Four nontoxigenic *C. botulinum* group II isolates were studied and cardinal parameter values for  $\text{CO}_2$  ( $\text{CO}_{2\text{max}}$  in equilibrium = 280.75%) and phenol ( $P_{\text{max}} = 27.52$  ppm) were determined in seafood challenge studies and used to expand an available model. The new model included the effect of ten environmental factors (temperature, pH,  $a_w$ , acetic, benzoic, citric, lactic and sorbic acids,  $\text{CO}_2$  and phenol).

**Results:** Evaluation of the new model by comparison of observed and predicted  $\mu_{\text{max}}$ -values for 56 growth curves in seafood resulted in bias factor of 1.12 and accuracy factor of 1.40. Interestingly, smoke components (phenol) in hot-smoked fish, opposed to cold-smoked fish, had no inhibitory effect on growth of non-proteolytic *C. botulinum*.

**Conclusion and Relevance:** The new and expanded model can be used to facilitate product development for a wide range of chilled seafood. As an example for chilled ( $7^{\circ}\text{C}$ ) cold-smoked halibut with pH 6.3, 15 ppm phenol and 3500 ppm acetic and 7000 ppm lactic acids: Reducing WPS from 3.5% to 1.5% resulted in predicted growth ( $\psi$ -value = 0.69). However, with 5500 ppm acetic and 9000 lactic acids at pH 6.0 growth of non-proteolytic *C. botulinum* was prevented ( $\psi$ -value = 1.56). For vacuum-packed cold-smoked salmon at  $5^{\circ}\text{C}$ , with pH 6.2, 7000 ppm lactic acid and 10 ppm phenol: When WPS was reduced from 3.5% to 1.5%, growth was predicted ( $\psi$ -value = 0.63). When using MAP with 50%  $\text{CO}_2$  in equilibrium, 3000 ppm acetic acid and pH 5.8 growth was prevented ( $\psi$ -value  $> 2$ ) as for 3.5 % WPS.

Keywords: Phenol; low salt; carbon dioxide; MAP; chilled seafood

### 39: NATURAL COMPOUND TO CONTRAST THE GROWTH OF *CLOSTRIDIUM PERFRINGENS* IN PORK MEAT, PERFORMANCE OF PREDICTIVE TOOLS

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**Introduction:** *Clostridium perfringens* is a spore-forming pathogen that may cause foodborne illness due to the ingestion of contaminated meat improperly cooled. Sodium nitrite was usually used as antimicrobial to control the growth, but nowadays the production of "clean-label" foods made with natural compound is increasing. Objectives of this study were a) to evaluate the effect of sodium nitrite and natural compounds on spore out-growth in meat during two linear cooling profiles and b) to evaluate the performance of two predictive tools.

**Methodology:** Two batches of minced pork meat was inoculated with *C. perfringens* spores and four groups of samples were prepared: meat (G1); meat with brine solution (S) with 1 % of NaCl (G2); with S and 1% of natural compound (G3), with S and 0.015 % of sodium nitrite (G4). Each sample (100 g) was vacuum packed, cooked at 75°C for 20 min and then cooled from 55°C to 7°C in 15 h or in 21 h. Observed data (Log CFU/g) was compared with those predicted by Perfringens Predictor (PP) and Pathogen Predictive Model (PPM). Accuracy (*Af*) and Bias factors (*Bf*) were used to evaluate the performance of the models.

**Results:** After 15 h of cooling the pathogen mean net growth were 5.9 (G1), 4.4 (G2), 4.1 (G3), 0.4 (G4) Log UFC/g, while after 21 h the mean net growth were 6.2 (G1), 5.4 (G2), 5.1 (G3), 1.1 (G4) log UFC/g. In meat without nitrites, predicted and observed concentration was practically overlapping. For PP *Af* ranged from 1.02 to 1.05 and *Bf* from 0.98 to 0.99; for PMP *Af* from 1.03 to 1.06 and *Bf* from 0.97 to 0.99. While, in meat with nitrites the predictive models overestimate the pathogen concentration. For PP *Af* ranged from 1.74 to 1.87 and *Bf* from 0.66 to 0.68; for PMP *Af* ranged from 1.60 to 1.65 and *Bf* from 0.72 to 0.74.

**Conclusion and Relevance:** Natural compound doesn't contrast the growth of *C. perfringens* in meat such nitrites do. Predictive model are useful tools; food processors and regulatory agencies can use these instruments to evaluate the safety of cooked meat.

Keywords: Spore forming bacteria; cooling profile; nitrites; clean-label; food safety

### 53: A SIMPLE CORRELATION BETWEEN THE CARDINAL TEMPERATURES OF *BACILLUS CEREUS* SENSU LATO

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<sup>5</sup>Nestlé Research Center, Lausanne, Switzerland

**Introduction:** A number of stochastic models have been proposed for QMRA purposes based on the characterisation of the growth of individual strains within a single species. In these models, the distributions of the cardinal temperature parameters (e.g. minimum, optimum and maximum temperature for growth) are usually assumed to be independent. However, correlations between these cardinal temperatures have been highlighted in a number of studies. In a recent publication, a correlation was also highlighted between the minimum temperature of 12 strains of *Bacillus cereus* and the b-slopes of the Ratkowsky temperature growth model.

**Methodology:** The objective of this study is to highlight correlations between the cardinal temperatures of *Bacillus cereus* sensu lato strains. The data set consists of cardinal temperatures values identified for over 40 strains belonging to 6 different phylogenetic groups (group II to VII). The correlation was established using a subset of 20 strains, the remaining subset being used for validation.

**Results:** The training data set was found to exhibit a simple linear relationship between the cardinal temperatures of *Bacillus cereus* strains ( $R^2=0.90$ ). This relationship makes it possible to deduce one of the cardinal temperatures (e.g.  $T_{max}$ ) from the two other cardinal parameters (e.g.  $T_{min}$  and  $T_{opt}$ ). The fitted model successfully describes the correlations between cardinal temperatures for the strains not used for the fitting. Unexpectedly, it has also provided an accurate description of existing correlations for some strains of *Bacillus amyloliquefaciens*, *Bacillus subtilis* and *Bacillus licheniformis*, specially when their optimal temperature fall below 45°C. For several individual strains of *B. cereus*, the relationship between  $\mu_{max}$  and temperature was successfully predicted from 2 of their cardinal temperatures only and the existing correlations between the growth model parameters (b-slopes of the Ratkowsky model and  $T_{min}$ ,  $T_{opt}$  and  $T_{max}$ ).

**Conclusion and Relevance:** This study shows that strong correlation between the cardinal temperature parameters exist, that should be taken into account for stochastic simulations.

**Keywords:** *Bacillus cereus*; cardinal temperatures; strain variability; correlation

## 59: GROWTH OF *ESCHERICHIA COLI* AND *CRONOBACTER SAKAZAKII* IN MINIMALLY-PROCESSED FRESH-CUT FRUITS: MODELLING THE EFFECT OF TEMPERATURE

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**Introduction:** *Cronobacter sakazakii*, is a ubiquitous bacterium associated to plants isolated from foods such as powdered infant formula and plant origin ready-to-eat food as well as from industrial or home environments. It is an opportunistic pathogen to infants, neonates and vulnerable adults. The pathogenic strains of *Escherichia coli* are among the most relevant etiologic agents of food borne diseases. The growth of *E. coli* and *C. sakazakii* populations in inoculated fresh-cut fruits incubated at different temperatures was studied in ‘Royal gala’ apple, ‘Rocha’ pear, ‘Piel de sapo’ melon, (Santo et al. 2016), and ‘Tommy Atkins’ mangoes Santo et al. 2018). The objective of the present study was to expand previous studies by using “secondary models” (sensu Ross and Dalgaard, 2004) in an attempt to describe the effect of environmental, storage conditions, viz. temperature (4°C, 8°C, 12°C and 20°C), on the values of the parameters of “primary models” of growth.

**Methodology:** Microbial growth parameters of *E. coli* and *C. sakazakii*, namely growth rate ( $\mu_{max}$ ), adaptation period (lagt) and the final microbial populations’ abundance ( $\log N_{max}$ ) estimated for minimally-processed fresh-cut apple, pear, melon and mangoes were compiled. Square-root-type models and models based on the Arrhenius equation were tentatively fitted to the data using R. “Secondary models” goodness of fit was assessed using RMSE and  $R^2$ . These (linear) models were compared among fruits using ANCOVA.

**Results:** The relationships between  $\mu_{max}$  and  $\log N_{max}$  of *E. coli* and *C. sakazakii* and the incubation temperatures were significantly ( $p < 0.05$ ,  $R^2 > 0.93$ ) fitted by both square-root-type models and models based on the Arrhenius equation in apple, pear and melon but not in the case of mango. There were significant ( $p < 0.05$ ) differences in parameters of “secondary models” among fruits. In contrast, several “primary models” of growth at the temperatures of incubation tested did not include parameter lagt because no adaptation period was observed.

**Conclusion and Relevance:** Thus, we were unable to fit “secondary models” to the relationship between lagt and incubation temperature. The methodological approach and the findings are discussed in light of published literature.

**Keywords:** *Cronobacter sakazakii*, *Escherichia coli*, fresh-cut fruits, secondary models, temperature

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## 72: PROBABILISTIC MODELLING OF BACTERIAL RESPONSES UNDER THE EFFECTS OF SELECTED CONCENTRATIONS OF THYME ESSENTIAL OIL OR THYMOL IN VAPOR PHASE

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**Introduction:** Consumers demand for more natural products and clean labels has increased in recent years; therefore, there is an increasing need to find natural sources of food preservatives, such as herbs and spices, or their extracts and essential oils (EOs).

**Methodology:** In this work, logistic regression was utilized to describe and predict the vapor phase antibacterial effect of thyme (*Thymus vulgaris*) EO or thymol (one of the major components of this EO) against three bacteria (*Listeria monocytogenes*, *Salmonella enterica* Typhimurium, or *Pseudomonas fluorescens*) at selected pHs (6.0 or 6.5) and temperatures (10, 15, 25, or 35 °C). Thyme EO and thymol antibacterial activity was evaluated using the inverted Petri dish technique; volumes tested varied from 5 to 1200  $\mu\text{L}/L_{\text{air}}$ , depending on tested bacteria, pH, and temperature. Data sets for the probabilistic model were obtained experimentally from those conditions of pH, temperature, and concentration (of thyme EO or thymol) that caused bacterial growth or no growth after incubation. When growth was observed a value of 1 was assigned while a 0 value was utilized when no-growth was observed (representing a 6-log reduction). Developed models, by varying studied conditions, were used to predict the probability of bacterial growth and to calculate the critical values of thyme EO or thymol concentrations needed to inhibit bacterial growth with selected probabilities.

**Results:** Results demonstrated that thyme EO or thymol concentration, pH, and incubation temperature, as well as the interaction between temperature and concentration, significantly ( $p < 0.10$ ) affected growth of the three studied bacteria. Increasing thyme EO or thymol concentration, gradually affects the probability of growth of each bacteria. With the obtained models, critical concentrations of thyme EO or thymol necessary to reduce the probability of growth (0.05) were calculated. As temperature and pH increased, the preservative concentration needed for the inhibition of *S. enterica* and *P. fluorescens* also increased. Selected predicted combinations obtained by the logistic model were validated experimentally.

**Conclusion and Relevance:** Logistic regression was useful to model adequately the effects of combined tested preservation factors on studied bacterial growth. Thus, can be utilized to determine the microbial growth/no growth boundary when different microbial stress factors are combined.

**Keywords:** Probabilistic modelling; thyme essential oil; thymol; vapor phase; logistic regression

## **78: MODELLING *WEISSELLA VIRIDESCENS* GROWTH IN HAM VACUUM-PACKED WITH ACTIVE FILMS OF CELLULOSE ACETATE-CARVACROL**

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**Introduction:** Antimicrobial packaging is an emerging technology to extend food shelf-life by a prolonged release of active compounds on the food surface, where most of the microbial spoiling occurs, promoting the antimicrobial effect with low amounts of the active compound. This study aimed to evaluate the *W. viridescens* growth in ham slices using an active film of biodegradable cellulose acetate (CA) containing carvacrol, the main compound of oregano essential oil.

**Methodology:** Films were produced by casting commercial CA (degree of substitution = 2.2 and  $M_w = 58000$  g/mol) with up to 10% of carvacrol in mass. The ham was sterilized, sliced, and inoculated with 100  $\mu$ L of the *W. viridescens* ( $10^4$  UFC/mL). Then, the active films were placed over the ham slices and stored under vacuum in plastic bags at 8 °C. For evaluating bacterial growth, sequential 10-fold dilutions were performed in peptone water (1% w/v), 1 mL was transferred to a Petri dish, and was added a double layer of MRS agar. The plates were incubated at 30 °C for 48 h followed by counting the viable cells. The Baranyi and Roberts model described the *W. viridescens* growth.

**Results:** The model showed a good fit to the experimental growth data of *W. viridescens*, since the  $R^2$ , Bias- and accuracy-factor were close to 1. The presence of the active film decreased the maximum specific growth rate from 0.037 to 0.014  $\text{h}^{-1}$ , decreased the maximum population observed from 8.32 to 7.15 log[CFU/g], and increased the lag phase from 12 to 66 h when comparing to vacuum-packed ham without the CA-carvacrol-film. It was observed a potential extension on the shelf-life, assumed as the time to reach 7 log[CFU/g], in 200% (from 102 h to 307 h).

**Conclusion and Relevance:** CA-carvacrol-films can extend the shelf-life of meat products, such as sliced ham, by delaying the growth due to increasing the adaptation time and reducing the specific growth rate. The active CA-films with carvacrol can contribute to reducing the use of non-biodegradable polymers and therefore plastic waste, besides decreasing food waste and the addition of chemical preservatives to food products.

**Keywords:** Biodegradable polymer; antimicrobial; shelf-life; Baranyi and Roberts model

## **79: MODELLING GROWTH AND SURVIVAL OF *SALMONELLA ENTERICA* TYPHIMURIUM UNDER OSMOTIC STRESS CONDITIONS**

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**Introduction:** Controlling the presence of pathogenic bacteria, such as *Salmonella* spp., is a constant challenge for the food industry. *Salmonella* spp. is one of the main concerns, once it can survive under conditions that are unfavorable for its growth, such as under relatively low water activity ( $a_w$ ).

**Methodology:** The effect of the osmotic stress and the physiological state of *Salmonella enterica* Typhimurium (ATCC 14028) cells were evaluated by growing inocula containing exponential and stationary phase cells obtained at 25 and 35 °C. Solutions with 100 mL of brain heart infusion broth (BHI) and 7% (w/v) of NaCl, reaching  $a_w$  of 0.95, were prepared and inoculated with the microbial suspensions of *S. Typhimurium* (around  $10^4$  CFU/mL). The growth curves were obtained by viable counts. The primary Baranyi and Roberts model was adjusted to the data, using a nonlinear regression method in the Microsoft Excel software (solver supplement).

**Results:** During exposure at a low  $a_w$  condition, the kinetic of growing was characterized by a decrease in the initial cell counts followed by an exponential increase, the so-called Phoenix phenomenon. Baranyi and Roberts model was adapted based on the assumption that the log count curve of the total population was the sum of a dying and a surviving-then-growing subpopulation. The predictive ability of the model was assessed through statistical indexes (bias factor 1.016, 1.000; accuracy factor 1.178, 1.034; RMSE 0.288, 0.110;  $R^2$  0.973, 0.993 for cells in the exponential phase at 25 °C and stationary phase at 35 °C, respectively), showing safe predictions. Our results showed that the responses of the bacteria cells varied with the physiological history of the inoculum. Comparing the two inoculum conditions evaluated, the use of the inoculum in the stationary phase at 35 °C of *S. Typhimurium* led to an increase in the lag phase and in the maximum specific growth rate.

**Conclusion and Relevance:** The physiological state of the inoculum is decisive for the behavior of *Salmonella* Typhimurium cells. The complexity of bacterial response close to the growth limits should be better evaluated to obtain trustful parameters for the predictive models.

**Keywords:** Food safety; survival; physiological state; Phoenix phenomenon



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## 81: MODELLING OF *LISTERIA MONOCYTOGENES* GROWTH IN CHICKEN NUGGETS AS A FUNCTION OF TEMPERATURE

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**Introduction:** Due to its ubiquitous nature, *Listeria monocytogenes* may constitute a post-processing contaminant of breaded poultry products. The objective of the present study was the quantitative description of *L. monocytogenes* growth in chicken nuggets as a function of temperature.

**Methodology:** Chicken nuggets (breaded chicken breast cuts) were inoculated with *L. monocytogenes* (ca. 10 CFU/g) and stored under different isothermal conditions (4, 8, 12 and 16 °C) for a maximum period of 20 days. Two independent experimental replicates were conducted and duplicate chicken nuggets were analyzed at regular time intervals during storage within each replicate. The growth kinetic parameters of the pathogen were estimated at each one of the studied temperatures using the primary model of Baranyi and Roberts, and the effect of temperature on the maximum specific growth rate ( $\mu_{\max}$ ) was modeled using a square-root-type model. The secondary model was evaluated against independent growth data (external validation), which were generated during storage of inoculated chicken nuggets under both isothermal (10 °C) and dynamic temperature (periodic temperature changes from 4 to 12 °C) conditions. The spoilage microorganisms of the product under the conditions of this study also were determined.

**Results:** The mean ( $\pm$  standard deviation,  $n=4$ )  $\mu_{\max}$  values ( $\text{h}^{-1}$ ) of *L. monocytogenes* in chicken nuggets stored at 4, 8, 12 and 16 °C were estimated to be 0.036 ( $\pm$  0.007), 0.069 ( $\pm$  0.016), 0.103 ( $\pm$  0.030) and 0.180 ( $\pm$  0.059), respectively. The estimated value of  $T_{\min}$  was -5.80 °C. Furthermore, as indicated by the external validation results, the developed model exhibited a satisfactory performance in describing the effect of storage temperature on the pathogen's growth. With reference to the dominant spoilage microflora of chicken nuggets, this was determined to consist of psychrotrophic yeasts, with the latter exhibiting a rather competitive activity against *L. monocytogenes* growth, particularly during storage at 4 °C.

**Conclusion and Relevance:** The results of this study should be useful in the exposure assessment part of quantitative microbiological risk assessment regarding breaded chicken products.

**Acknowledgements:** This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-04344).

**Keywords:** Chicken nuggets; growth; *Listeria monocytogenes*; modelling; temperature

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## 89: PREDICTING THE EFFECT OF SALT ON HEAT TOLERANCE OF *LISTERIA MONOCYTOGENES* IN MEAT AND FISH PRODUCTS

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**Introduction:** *Listeria monocytogenes* is a potentially fatal foodborne pathogen that can be found in various food products, including meats. It can tolerate adverse conditions such as high salt concentrations, thus, the elimination of the organism is difficult in food processing. This study was conducted to enable prediction of the effect of salt on heat tolerance of *L. monocytogenes* in food.

**Methodology:** The experimental work involved minced pork, chicken and salmon samples with different water phase salt (WPS) concentrations from 0 to 5.6 % inoculated with late-stationary phase *L. monocytogenes* cultures from three strains associated with fish, meat and industrial environment, respectively. Samples were vacuum-packed in sterile bags, immersed in water bath, and held at different constant temperatures from 57 to 65 °C. Heat tolerance was defined as the time to achieve 3 decimal reductions, *i.e.* 3D-values, by using both log-linear and non-linear-regression models. Subsequently, development of a secondary predictive model describing the combined effect of temperature and salt on heat tolerance of *L. monocytogenes* was carried out. The mathematical structure of the model was adopted from previous studies. Three different approaches, denoted i) Log-linear, ii) GlnaFiT and iii) F-test, were applied for selection of the 3D-values to be included as response in the development of the model followed by a validation process of the most accurate of those three approaches.

**Results:** The study showed that the most heat tolerant strain was the one from industrial environment. Heat tolerance increased with up to 3-fold for 3 % WPS and up to 5-fold for 5.6 % WPS depending on *L. monocytogenes* strain and heating temperature.

**Conclusion and Relevance:** The F-test approach provided the best model by estimating model parameters with the lowest  $RSS = 0.257$ . Validation was executed by including independent log3D-values from nine different studies. The bias factor and accuracy factors were used for evaluating model performance.  $Bf = 1.22$  and  $Af = 1.33$  implied that the model was acceptable with room for improvement. The model obtained from this study enables food processors to design proper thermal processes to eliminate *L. monocytogenes* in meat and fish products to ensure safety and prevent foodborne listeriosis.

**Keywords:** Food safety; heat treatment; meat; fish; salt

## 92: SURVIVAL OF *SALMONELLA ENTERICA* SER. ENTERITIDIS IN STRAWBERRIES DURING STORAGE AT DIFFERENT TEMPERATURES

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**Introduction:** Strawberries can be susceptible to microbial contamination after harvesting by enteric pathogens such as *Salmonella* spp., being an emerging risk for public health. As strawberries can be stored at a wide temperature range, the risk of *Salmonella* survival in contaminated fruits is not negligible. In this study, the survival kinetics of *Salmonella* was studied during storage at different temperatures. Predictive models were fitted to observed data to estimate the inactivation rates at the studied conditions.

**Methodology:** *Salmonella enterica* ser. Enteritidis strain was spot inoculated on fresh strawberries considering an initial concentration of 4 log CFU/g. Temperature of storage and relative humidity were considered as external variables. After drying for 1h in a flow cabinet, strawberries (50 g/sample) were stored aerobically in plastic containers at 20°C, 7°C y 4°C until the end of the shelf-life. Survival kinetics was modelled through Weibull, and log-linear inactivation models included in the Bionactivation package in R. Univariate analysis ANOVA was carried out for temperature to evaluate the effect of different external variables on the inhibition of *S. Enteritidis* with a Tukey post-hoc test ( $p < 0.05$ ).

**Results:** The microbial population of *S. Enteritidis* declined at 20°C until 72 h where spoilage of strawberries caused by moulds' growth were observed. At refrigeration temperatures, a reduction of approx. 2 log CFU/g was observed at the 8<sup>th</sup> day of storage. No significant differences were observed in pH values, with a mean of 3.7 ( $p > 0.05$ ). Weibull and log linear models presented a good adjustment to observed data, most them having  $R^2$  values  $> 0.9$  (MSE  $< 0.50$ ). Storage of strawberries at ambient temperature produced an increased survival of *S. enterica* since significant differences were obtained for the inactivation rates ( $p < 0.05$ ) in comparison to the studied refrigeration temperatures.

**Conclusion and Relevance:** It is concluded that temperature control used as a single factor cannot guarantee *Salmonella* inhibition in contaminated strawberries. Additional preventive measures at primary, transformation, distribution and consumption steps should be implemented to eradicate pathogenic contamination in strawberries. This study could be applied by food processors and risk managers to set harmonized temperature and time conditions along the strawberries processing chain.

**Keywords:** Strawberries; *Salmonella*; temperature; fruit

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## 105: MODELLING THE NON-THERMAL INACTIVATION OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* DURING MATURATION AND SHELF LIFE OF CHEESES

Fanny Tenenhaus-Aziza<sup>1</sup>, Janushan Christy<sup>2</sup>, Valérie Michel<sup>2</sup>, Louis Coroller<sup>3</sup>

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**Introduction:** Being able to assess the slow decline of pathogenic populations during cheese maturation has several advantages (rational explanation of the microbial behavior, justification of the “hurdle” technology, optimisation of sampling plan, determination of shelf life, experimentation economy, etc.). Coroller et al., 2012 (*Coroller, L., Kan-King-Yu, D., Leguerinel, I., Mafart, P., and Membré, J.-M. (2012). Modelling of growth, growth/no-growth interface and nonthermal inactivation areas of Listeria in foods. International Journal of Food Microbiology 152, 139–152*) modelled the inactivation of *Listeria monocytogenes* and *Salmonella*, due to acid stress, related to the concentration of organic acids present in the bacterial environment. The objective of this study was to validate the relevance of this model on Shiga toxin-producing *Escherichia coli* (STEC) behavior during cheese ripening and distribution.

**Methodology:** Several kinetics generated by an acidic environment on STEC populations were collected. They allowed to estimate the parameters characterising the resistance of STEC strains to acid stress (including MICs for different serotypes of STECs), and their sensitivity to variations in pH and organic acid concentration, at different temperatures levels. The ranges of studies for the selected factors were between 8 to 15°C for the temperature, between 5 and 7 for the pH and between 0.5 and 1% for the lactic acid. Lactic acid was the only one for which we had sufficient data on its evolution during cheese maturation. The activity of water was not studied, because the  $a_w$  values measured in cheeses are generally high and stable, between 0.96 and 0.98.

**Results:** Parameter values acquired experimentally for STEC were then integrated into the non-thermal inactivation models of Coroller et al. (2012). Finally, the simulation results were confronted with experimental challenge test data of STEC in a long-ripened raw milk cheese.

**Conclusion and Relevance:** The model was validated and can now be used in wider quantitative risk assessment models. In the future, the impact of other organic acids (propionic acid, acetic acid) could be studied in a similar way.

**Keywords:** Non-thermal inactivation model; lactic acid; minimum inhibitory concentration; resistance parameters

## 109: HEAT RESISTANCE OF *LISTERIA MONOCYTOGENES* IN VANILLA AND STRAWBERRY ICE CREAM SYRUPS

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**Introduction:** *Listeria monocytogenes* is a psychotrophic bacterium that has caused foodborne outbreaks due to ice cream consumption. Despite this, there is a lack of data about *L. monocytogenes* resistance during thermal process of ice cream. Therefore, the aim of this work was to determine the inactivation kinetic parameters of *L. monocytogenes* during ice cream syrup (ICS) pasteurisation.

**Methodology:** The inactivation kinetic was studied on vanilla (VICS; pH=7.2; 36.7°Brix) and strawberry ice cream syrups (SICS; pH=6.0; 35.2°Brix). Firstly, a cell suspension of three strains of *L. monocytogenes* serovar 1/2b, previously isolated from ice cream, was prepared. Both ICS were inoculated with this suspension ( $10^7$  CFU/g) and then heat at 60, 65 and 70°C, employing a coated stainless-steel reactor coupled to a thermostatic bath and a mechanical stirrer. Samples were taken at time intervals for enumeration of survivors using TSA-YE, following incubation at 30°C/48h. The number of survivors (logCFU/g) were plotted against time process and inactivation models were fitted to data using Ginfat<sup>®</sup>. D and z-values were estimated. ANOVA followed by t-tests were employed to assess significant differences regarding inactivation parameters.

**Results:** For both ICS, *L. monocytogenes* showed a log-linear inactivation kinetic ( $R^2 > 0.9$ ). For VICS and SICS, at 60, 65 and 70°C, D-values were 9.72, 1.40, 0.31 min and 5.66, 0.93, 0.19 min, respectively. D-values of *L. monocytogenes* were always lower on SICS than on VICS ( $p < 0.05$ ), likely reflecting SICS lower pH compared to VICS. The z-values for *L. monocytogenes* were 6.7°C (VICS) and 6.8°C (SICS), respectively.

**Conclusion and Relevance:** The data found indicate a low thermal resistance of *L. monocytogenes* on ICS. Considering that ICS are pasteurised at 68.3°C/30 minutes or 79.4°C/25 sec, these data suggest that the incidence of *L. monocytogenes* on ice cream may be related to post-pasteurisation contamination. The determination of inactivation parameters is a great importance for thermal processing design to guarantee the pathogens reduction in raw materials and ingredients. These data are of key relevance for the conduction of risk assessment studies, which can be further used to generate scientific supported evidences to enhance ice cream safety and protect public health.

**Keywords:** Thermal processing; D-value; food safety; thermal inactivation; ice cream processing

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### 137: OPTIMISATION BY SURFACE RESPONSE METHODOLOGY OF THE COMBINED EFFECT OF TEMPERATURE AND INITIAL SUGAR CONCENTRATION ON THE KINETIC GROWTH BEHAVIOUR OF *SACCHAROMYCES FIBULIGERA* BIOMASS, CELL-WALL AND CELL-WALL FRACTIONS PRODUCTION.

Paula García Oliveira<sup>1</sup>, Antía González Pereira<sup>1</sup>, Cecilia Jiménez López<sup>1</sup>, Catarina L. Lopes<sup>1</sup>, Lillian Barros<sup>2</sup>, Miguel Ángel Prieto<sup>1,2</sup>, Isabel C.F.R. Ferreira<sup>2</sup>, Jesús Simal-Gándara<sup>1</sup>

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**Introduction:** Spain is the largest producer of mussels in Europe due to aquaculture activities, located specially in the Northwest. The mussel processing industries release effluents that are rich in organic compounds that have been proved to be a suitable growth medium for microorganisms for industrial application. Numerous empirical evidences have demonstrated that oligosaccharides and  $\beta$ -glucans present in the cell wall (*cw*) of microorganisms produce healthy effects such as antitumor and immunomodulation agents. Authors have shown that *Saccharomyces fibuliger* yeast strain has an enormous potential for *cw* production. Many scientific studies focus on the biomass (*b*) yield production, rather than in relation to the *cw* thickening during kinetic cell growth and the conditions that maximise it. Studies suggested that the temperature, pH, and initial substrates concentrations alters the cell-wall/biomass (*cw/b*) yield. This study aims to improve the production of *cw* in relation to the *Saccharomyces fibuliger* growth.

**Methodology:** Dilutions of mussel process wastewaters with different sugar concentration were inoculated with *Saccharomyces fibuliger* inocula. After the *cw* extraction and purification, the sigmoid profiles were carried out and the maximum growth rate and the lag-phase were calculated and used as experimental results in further analytical solutions. Then, the parametric values were used to find the best possible interactions between variables, response surface methodology (RSM) was used to predict experimental conditions that maximise *cw/b* yield production.

**Results:** Temperature (*T*) and initial sugar concentration (*S*) were found the only relevant variables, and a clear optimum was located at 25.6 g/L and 28 °C, respectively. Under such conditions, the expected production of *cw/b* was 0.635 g/g. The high value of the adjusted determination coefficient ( $R^2_{adj}=0.986$ ) and the no significant difference ( $P>0.05$ ) between predicted and experimental values demonstrated the validity of the optimisation model proposed.

**Conclusion and Relevance:** Understanding the joint effect of these variables on the growth and *cw* yield production is crucial; and, to our knowledge, it has not been reported. This study aims to improve the production of *cw*, rich in bioactive compounds, in relation to the *Saccharomyces fibuliger* growth. By means of RSM, the joint effect of temperature and initial sugars on culture media was studied and the *cw* yield production was maximised.

Keywords: *Saccharomyces fibuligera*; cell-wall production; bioactive compounds; process optimisation

### 30: DEVELOPMENT OF INTELLIGENT PACKAGING SOLUTIONS TO INCREASE RESOURCE EFFICIENCY IN COLD SUPPLY CHAINS

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**Introduction:** Alongside supply chains of perishable food products, rejections and food waste occur due to lack of information at different chain levels. Intelligent packaging solutions, such as Time-Temperature-Indicators (TTIs), enable the estimation of the remaining shelf life at all steps of the supply chain by providing information on the actual product status. However, specific requirements for different supply chains, a digital readout and data exchange via traceability systems do still not exist in current intelligent packaging systems. The aim of the project “Intelli-Pack” (2018 – 2021) is the development and implementation of an innovative intelligent packaging system to increase the quality, safety and resource efficiency of perishable products.

**Methodology:** Laboratory studies for the modelling of spoilage kinetics of raw sausage, fish, galeeny breast and fresh-cut-salad are conducted. Established intelligent systems are improved by developing a smartphone application combined with a database for the digital readout of the TTI colour change and the integration of information as decision guidance for stakeholders, including consumers, to optimise logistic processes. Legal aspects accompanied by the implementation of intelligent packaging and a flexible shelf life are investigated. Pilot studies are conducted to validate the advanced packaging systems adjusted to different supply chains (B2B, B2C, online). Based on the project results, an online support tool is developed to provide information about intelligent packaging solutions for all participants to optimise cold chain management. Additionally, an assessment tool for sustainability and resource efficiency of intelligent packaging is integrated into the online platform.

**Results:** First results of laboratory studies for the modelling of spoilage of sausages will be presented as well as a first smartphone application for the digital readout of the TTI colour change. An experimental setup to customize the parameters to predict the remaining shelf life on the base of measured TTI values will be discussed.

**Conclusion and Relevance:** The project provides an integral system, considering specific requirements of all stakeholders along the supply chains B2B, B2C and online trading. It obtains the implementation of dynamic shelf life and dynamic pricing combined with a

digital implementation of intelligent packaging under the aspects of logistics, traceability and sustainability.

Keywords: Intelligent packaging systems; food logistics; supply chain management; traceability; sustainability

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### **31: COMBINING PREDICTIVE SHELF LIFE MODELS WITH RAPID METHODS IN MEAT SUPPLY CHAINS**

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**Introduction:** Due to the lack of real-time information about the microbial load on meat surfaces, the applicability of predictive shelf life models and Process Analytical Technology (PAT) in the meat industry is still challenging. Thus, the aim was to enhance the significance of shelf life models by the application of spectroscopic sensors as tool for a real-time determination of the initial microbial load on different types of meat.

**Methodology:** To define the relevant parameters for the shelf life models, the spoilage process was analysed under different constant temperature conditions. Therefore, storage tests with chicken breast filets and different cuts of pork were conducted and the initial quality was evaluated. The total viable count (TVC) and typical spoilage organisms were determined by classical microbial investigations. Further quality parameters such as colour, sensory attributes and pH were also analysed. The shelf life models were established by using the modified Gompertz and Arrhenius equation. In addition, spectroscopic measurements were carried out on different processing levels and cuts of meat in parallel to classical cultural microbial analysis to validate the accuracy, robustness, and reliability of the method.

**Results:** The results of the microbial analysis showed a high variability in the initial quality of the products in the range of 1.5 - 4.9 log cfu/cm<sup>2</sup> for pork and 1.9 - 4.1 log cfu/cm<sup>2</sup> for chicken. The validation of the rapid method for the determination of TVC showed that the accuracy and robustness depends on the type, processing level and cut of the meat. However, it is a promising tool for the determination of the TVC on fresh meat within seconds. The results delivered by spectroscopy in combination with the predictive models showed the potential for the calculation of shelf life in real-time.

**Conclusion and Relevance:** The information delivered by the spectroscopy and the predictive models could be integrated into an early warning tool by using PAT. This offers an on-line feedback for process adjustment and supports decision making during



production, storage management and logistic processes, which leads to an overall reduction of food waste in meat chains and contributes to improve the quality and safety.

Keywords: Predictive microbiology; rapid methods; spectroscopy; meat spoilage; shelf life models

### 35: POLYPHENOLOXIDASE AND PEROXIDASE KINETIC INACTIVATION IN COCONUT WATER UNDER DYNAMIC THERMAL AND THERMOSONICATION PROCESS

Mariana Matos Ribeiro<sup>1</sup>, Tayná Márcia Teixeira Ferreira<sup>1</sup>, Vasilis Valdramidis<sup>2</sup>, Vanessa Rios de Souza<sup>1,3</sup>

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**Introduction:** In order to minimize the degradation of coconut water quality caused by the conventional heat treatment, non-conventional preservation methods have been studied. Previous studies demonstrated the great potential of the use of thermosonication for the processing of heat-sensitive products, such as coconut water due to its efficiency in enzymatic inactivation (polyphenoloxidase (PPO) and peroxidase (POD)). Additionally, ultrasound has less impact on quality properties (e.g., vitamins) of the treated products. Considering that most of the food processes are dynamic by nature, the implementation of approaches in which degradation kinetics are studied under dynamic processing conditions is imperative. These are expected to result in more accurate and precise parameter estimates. Therefore, the aim of the present work was to study the inactivation kinetics of PPO and POD in coconut water during dynamic thermal and thermosonication processes.

**Methodology:** The coconut water was submitted to 3 different thermosonication processes: 50%/15 min (UI=0.450 W/mL), 70%/10 min (UI=0.645 W/mL) and 90%/5 min (UI=0.640 W/mL). For each of the thermosonication treatments performed, a conventional thermal treatment with a temperature profile similar to that generated during thermosonication was carried out. The activity of PPO and POD was monitored throughout the treatment time. The kinetic parameters for thermosonication of coconut water were calculated by taking into account the unsteady-state conditions. Therefore, the degradation of PPO and POD was described based on a differential equation which introduces the parameters of the decimal-reduction ( $D_{ref}$  [min]), heat resistant constant ( $z_T$  [°C]) and ultrasound resistant constant ( $z_{UI}$  [W/mL]).

**Results:** Current results show that degradation kinetics could be estimated more accurately at the most intensive thermosonication conditions, i.e., 90%/5 min (0.640 W/mL), 70%/10 min (0.645 W/mL) that resulted in more than 50% reduction of the PPO and POD activity. PPO appeared to be more heat and less ultrasound resistant in relation to POD.

**Conclusion and Relevance:** The synergistic effect of heat and sonication could be expressed in relation to the degradation resistance of the PPO and POD enzymes. This

work highlights the importance of applying dynamic parameter estimation techniques for the accurate estimation of the thermosonication process parameters.

Keywords: Optimisation; dynamic conditions; enzymes; thermosonication

### **37: PHOTODYNAMIC INACTIVATION OF *BACILLUS CEREUS* SPORES: ESTIMATING THE INACTIVATION KINETIC PARAMETERS OF STRAINS FROM DIFFERENT SOURCES**

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**Introduction:** *Bacillus cereus* is a sporeforming bacteria commonly associated with foodborne diseases, being also able to spoil dairy products. As a sporeforming bacterium, *B. cereus* presents resistance to several inactivation treatments and because of that, alternatives to optimise its inactivation are frequently demanded. In addition, inactivation kinetics have been found to vary within strain and this study seeks to reveal the variability in PDI resistance of different *B. cereus* strains. The main aim of this work was to evaluate the inactivation of *B. cereus* strains by photodynamic inactivation (PDI).

**Methodology:** Preliminary tests with 12 strains of *B. cereus* isolated from different types of foods and outbreaks in Brazil were carried out in order to identify different clusters according to their PDI resistance at different concentrations of New Methylene Blue (NMB) using a light source of 96 LEDs (RED; 650 nm). Then, spore suspensions of 4 selected strains (more, medium and less resistant strains) were prepared and a further experiment was conducted with 5, 50 and 100 µM of NMB for up to 5 h in order to obtain the inactivation kinetic parameters (*D*-value and *z*-value) by PDI inactivation procedures.

**Results:** From the 12 strains pre-tested, 4 strains (B63, 436, B3 and 14579) were selected based on cluster analysis according to their PDI resistance. The least resistant strain to PDI was *B. cereus* 14579, for which 4.3 log reductions were observed after 20 min of light exposure, using 50 µM of NMB. PDI led to 4.7, 4.6 and 4.2 log reductions of strains B3, B63 and 436, respectively, after 5 h of light exposure, being considered the most resistant to PDI. The discrimination of *D*-values and *z*-values for the different strains will be presented.

**Conclusion and Relevance:** This work will allow gaining insights into the variability of *B. cereus* responses to PDI treatment as well as to the feasibility of using PDI for *B. cereus* control and future application in some types of food (e.g. cereals, seasonings, and dry fruits).

Keywords: PDI; *D*-value; *z*-value; *Bacillus cereus*; Variability

## **56: KINETIC MODELLING OF MICROBIAL INACTIVATION OF SMOOTHIES BY HIGH HYDROSTATIC PRESSURE**

Gerardo A. González-Tejedor, Alberto Garre, Asunción Iguaz, Alfredo Palop, Arantxa Aznar, Paula M. Periago, Francisco Artés-Hernández, Pablo S. Fernández

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**Introduction:** High Hydrostatic Pressure (HHP) is gaining popularity as a technology to substitute/complement thermal treatments for microbial inactivation for food preservation. However, in order to optimise these treatments, predictive models able to describe the microbial response to treatments of different intensities are required. The aim of this work was to find whether a predictive model would be useful to describe the inactivation of microorganism exposed to an HHP preservation process.

**Methodology:** Microbial inactivation experiments of *Listeria monocytogenes* were carried out in an HHP equipment at different pressure levels (300, 350, 400 and 450 MPa). A smoothie based on purple fruits was used. The results were modelled using the Geeraerd model for microbial inactivation. Furthermore, several quality attributes (sensorial quality, total soluble solids content, colour, pH, vitamin C and total phenolic content) were measured at different points of the treatments.

**Results:** The Geeraerd model was suitable for describing the microbial inactivation. For the lowest pressure level (300 MPa) substantial shoulder and tail effects were observed. For the remaining pressures tested, no shoulder was observed and at least 6 log-reductions were attained. Quality attributes were not affected by the treatment with respect to control (untreated) samples.

**Conclusion and Relevance:** HHP treatments with a pressure higher than 350 MPa are effective for inactivating *L. monocytogenes* in smoothies. They present a good alternative to thermal treatments due to their insignificant impact on the product quality and appropriate modelling can help to establish correct processing conditions. The predictive models tested in this investigation can be used by the food industry to optimise HHP treatments with the required duration and intensity. Moreover, the modelling approach applied here can be followed in other, similar, investigations.

**Keywords:** High hydrostatic pressure; smoothies; microbial inactivation kinetics; *Listeria monocytogenes*

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## Poster Session 2: Advances in Microbial Dynamics and Interactions; and Ensuring Safety of Traditional Foods

Time: 16:10 - 16:40

Date: 18th September 2019

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### 26: A MULTI-LEVEL ANALYSIS OF THE EFFECT OF FOOD MODEL SYSTEM MICROSTRUCTURE ON MICROBIAL DYNAMICS AND INTRA-SPECIES INTERACTIONS OF *LISTERIA* AND *L. LACTIS*

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**Introduction:** Minimal food processing techniques are of interest to replace traditional decontamination methods. However, they may induce an adaptive response in bacteria, affecting post-treatment survival and the potential for antimicrobial resistance (AMR) development. The chemical composition and rheological/structural properties of liquid and solid(like) foods varies greatly, which can affect a pathogen's growth kinetics and stress response. More specifically, cells on solid(like) surfaces are immobilised, and usually grow as 2D (flat) colonies. *Our previous work presented, for the first time, selective surface growth of Listeria on the protein phase of a complex biphasic protein-polysaccharide model system*<sup>1</sup>. Furthermore, differences exist in colony size/distribution for monophasic systems depending on growth type (surface/immersed) and viscosity, suggesting structural effects on a colony scale. This study investigates structural effects in a co-culture pathogen/microflora system, which can impact natural antimicrobial diffusion/efficacy and intra-species interactions and location, contributing to a changed environmental stress response, affecting their kinetics and potential AMR development.

**Methodology:** *Listeria* was grown planktonically (liquid TSB) or on the surface of a biphasic system (Xanthan gum, Whey protein), in monoculture with/without artificially added nisin, or in co-culture with *L. lactis* NZ9700 (nisin producer) or *L. lactis* NZ9800 (non-nisin producer), at 10°C, 30°C and 37°C. Microbial growth kinetics were monitored, and advanced microscopy techniques (confocal microscopy, scanning electron microscopy) were employed to quantify cellular interactions and spatial organisation on a colony level.

**Results:** Some system microstructural effects are observed on the microbial kinetics, with differences in monoculture/co-culture. Microscopy results show significant differences in spatial organisation and colony size, which can affect the environmental stress response. Furthermore, an extracellular polysaccharide matrix is observed between cells, suggesting biofilm rather than colony growth. For the first time, changing phase selectivity for all species when moving from mono- to co-culture has been observed.

**Conclusion and Relevance:** By combining macro- and microscopic techniques, this study provides insight into the environmental stress response/adaptation of *Listeria* grown on structured systems in response to novel processing technologies.

**References:** (1) Costello, K.M. *et al*, 2018. Int. J. Food. Microbiol. 286, 15-30.

Keywords: Nisin; co-culture; microscopy; *Listeria*; LAB

## 110: VARIABILITY OF BIOFILM FORMATION BY FIVE STRAINS OF *BACILLUS CEREUS* IN MONO AND CO-CULTURES ON STAINLESS STEEL AND POLYETHYLENE

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**Introduction:** *Bacillus cereus* biofilm is of major concern for the food industry. While most studies assess the biofilm formation in mono cultures, at industrial conditions, biofilms are composed by several microbial species. It is known that some species may impact on the ability of others to form biofilms. Therefore, this study aimed to assess the formation and variability of biofilms by five different strains of *B. cereus* in mono and co-cultures and in two different surfaces.

**Methodology:** Biofilm experiments were carried out with vegetative cells of five *Bacillus cereus* strains (CCGB 436, B3, B18, B51, and B94). Each strain was inoculated, separately, in stainless steel (SST) and polyethylene (PET) coupons. Coculture experiments, *B. cereus* strains (7 log<sub>10</sub>) were inoculated separately with each of the following microorganisms (7 log<sub>10</sub>): *Pseudomonas aeruginosa*, *Lactobacillus paracasei*, and *Staphylococcus aureus*. In addition, a further condition was studied in which each *B. cereus* strain was inoculated in co-culture with the three-mentioned species above. The inoculated coupons were incubated at 30 °C for 48 hours. Planktonic cells were counted on MYP (*B. cereus*); MRS (*L. paracasei*); Baird-Parker (*S. aureus*) and *Pseudomonas aeruginosa* (*Pseudomonas*) agars. For variability estimation, the coefficient of variation (CV) was calculated.

**Results:** The highest CV was observed in coculture *B. cereus* biofilm with *S. aureus* in PET coupons (42.51 %). Lowest CV was for *B. cereus* biofilm coculture with *L. paracasei* (8.13% in SST). Comparing biofilms monoculture of *B. cereus*, the higher variability was noted in SST coupons (31.2%). The *B. cereus* biofilm in coculture with *P. aeruginosa* showed variability in PET of 20.1%. The biofilm of all strains in coculture

showed CV of 31.71% in SST and 17.71% in PET. Overall, biofilms in SST coupons showed higher variability, excepting the biofilm of *B. cereus* in coculture with *L. paracasei* and in coculture with *P. aeruginosa*.

**Conclusion and Relevance:** Results suggest a high variability on the ability of *B. cereus* to form biofilms as affected by the surface and presence of other microorganisms. In addition, the microorganism species associated to the biofilm experiments was also found to impact on the variability of different strains of *B. cereus* to form biofilms.

**Keywords:** Heterogeneity; foodborne pathogens; *Pseudomonae*; *Lactobacillus*; *Staphylococcus*

### 136: MODELLING THE BIPHASIC GROWTH OF NON-STARTER LACTIC ACID BACTERIA IN THE PRESENCE OF STARTER CULTURE LYSATE

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**Introduction:** Since cheese is poor in energy for bacterial growth, it is believed that non-starter lactic acid bacteria (NSLAB) growth and flavour development is supported by nutrients from starter culture (SC) lysis. By testing this theory, biphasic growth (BG) is shown. The challenge of modelling BG has been reported. To apply primary models is essential to evaluate the impact of tested conditions on the stages of bacterial life cycle.

**Methodology:** A SC lysate was obtained by enzymatic digestion. The lysate was tested on 15 NSLAB strains found in cheese and two SC strains. Lactic acid bacteria (LAB) were individually inoculated (5 log cfu/mL) in M17 broth (no lactose), with or without 10% lysate, and incubated at 30°C/140h. The optical density (OD<sub>600nm</sub>) was modelled with the primary log-transformed Logistic model with delay and lag phase (I), growth rate ( $\mu_{\max}$ ) as well as maximum population density ( $N_{\max}$ ) were obtained. To deal with the lack-of-fit due to BG, the curves' data points were divided after graphic evaluation (GE) of the fitting at once and modelled in two-phases. The fitted data was analysed by GE and considering root-mean-square error (RMSE) values.

**Results:** As expected the growth of the two SC strains (also present in the lysate) was not affected by the lysate. However, 13 of the tested NSLAB strains such as *Lactobacillus rhamnosus* with OD values 0.30 (no lysate) and 0.50 (with lysate) had  $\mu_{\max}$  and  $N_{\max}$  increased. *Lb. delbrueckii* and *Lb. coryniformes* had a 3-h-shorter I on lysate. The BG growth was mostly shown on lysate. Lower RMSE values and better GE were obtained with this two-phases modelling approach.

**Conclusion and Relevance:** This approach described the BG of NSLAB on SC lysate, allowing comparison of the bacterial growth parameters. The effect of lysate was investigated and not all LAB were able to metabolize it. The degree of boost on NSLAB growth was species and strain dependent. Ripening of cheese is very dependent on bacterial interactions. An approach able to describe the dynamics of these interactions

allows the scientific community to investigate the mechanisms involved; and to support the industry in quality and safety improvement of cheese production.

Keywords: Biphasic growth; two-phase growth model; lactic acid bacteria; bacterial interaction; cheese quality and safety

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## **7: THE EFFECTS OF SUGAR CONCENTRATION, TEMPERATURE AND pH ON THE SURVIVAL OF *ESCHERICHIA COLI* ATCC 25922 IN THE TRADITIONAL LOCAL FOOD AMLOU**

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**Introduction:** Amlou spread is obtained by grinding roasted almonds mixed with honey or sugar and argan oil. Amlou is known as a specialty from the South of Morocco and it is characterised by an artisanal production chain in which microbial contamination may be promoted. In this study, a model was developed for predicting the survival of *Escherichia coli* ATCC 25922 in traditional Amlou to reduce such risks.

**Methodology:** A multi-level factorial design performed by Design expert software (version 11) was used to study the combined effect of three factors: sugar concentrations, pH and storage temperatures. For each combination of the environmental factors, the bacterial counts were modelled using the Baranyi model, as a function of time to estimate the kinetic parameters of *E. coli* death rate (DR) and survival period (SP).

**Results:** The results showed a reduction of more than 3 log units in all conditions with a high value of sugar concentration, which was found to have a significant effect on DR and SP. Data generated in the product contaminated with *E. coli* were used to develop linear models ( $p < 0.05$ ) with decimal logarithms of SP and DR as a function of three factors to predict the evolution of *E. coli* in Amlou. The coefficient of determination ( $R^2$ ) and root means square error (RMSE) obtained were, respectively, 0.75 and 1.38 for the death rate, and 0.67 and 0.59 for survival period.

**Conclusions and Relevance:** This study demonstrates the inactivation of *E. coli* in Amlou when sugar was increased. However, the indices of performance used showed that other factors such as argan oil and protein concentration should be considered in a more extensive study.

Keywords: Amlou spread; argan oil; survival period; death rate.

## **11: VALIDATION VIA CHALLENGE TEST OF A DYNAMIC GROWTH-DEATH MODEL FOR THE PREDICTION OF *LISTERIA MONOCYTOGENES* KINETICS IN *PECORINO DI FARINDOLA* CHEESE**

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**Introduction:** Predictive models are useful tools, foreseen by the European Union Food Law, for the evaluation of growth kinetics of pathogenic microorganisms in foodstuffs. Pecorino di Farindola is a typical cheese of Abruzzo Region (central Italy), produced according to traditional processing techniques. Aim of this study was to compare predictions obtained from a model, originally designed to predict the kinetics of *Listeria monocytogenes* in the dynamic growth-death environment of drying fresh sausage, with the results of challenge tests performed during the ripening of Pecorino di Farindola.

**Methodology:** Three batches of cheese were produced, artificially inoculated with a mixture of two *Listeria monocytogenes* strains and analysed. The raw milk used for cheese production was inoculated in order to get a concentration of  $10^5$  CFU/g. Sampling was carried out at fixed times (T = hours), being T0 the beginning of ripening. *Listeria monocytogenes* detection and enumeration were at T0, T144, T312, T480, T816, T1152, T1488, T1824, T2160, T2880 and T3576 (149th day of ripening). Temperature was 18°C during the whole ripening period; pH,  $a_w$  and lactic acid bacteria were also evaluated. Different predictive models were used in order to join growth and death patterns in a continuous way, including the highly uncertain growth/no growth range separating the two regions. A Mann-Whitney test was used to compare observed and predicted growth/death curves.

**Results:** In all batches, after a slight growth, a period of stability due to competition with microflora followed this initial situation. Then, a progressive inactivation was noted due to the reduction of water activity. Predicted microbial kinetics were satisfactory, as confirmed by the absence of statistically significant difference between observed and predicted curves ( $P > 0.05$ ).

**Conclusions and Relevance:** Predictive models can be used in food safety assessment, particularly in relation to legal requirements for *Listeria monocytogenes*. The present study proved, via challenge tests, that the dynamic growth/death model can be fruitfully used in cheese characterised by active competitive microflora and progressive drying during ripening.

**Keywords:** Challenge test; predictive model; validation; traditional cheese



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## 75: EFFECT OF YOGHURT STARTER CULTURE AND NICKEL OXIDE NANOPARTICLES ON THE ACTIVITY OF ENTEROTOXIGENIC *STAPHYLOCOCCUS AUREUS* IN DOMIATI CHEESE

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**Introduction:** Domiati cheese is the most popular type of white soft cheese in Egypt. *Staphylococcus aureus* (*S. aureus*) is a common microorganism which can easily contaminate Domiati cheese during processing and distribution. The aim of the present study was to model the inhibitory effect of yoghurt starter culture and nickel oxide nanoparticles (NiO NPs) on the development of the enterotoxigenic *S. aureus* (harbouring SEA gene), previously isolated from dairy products, together the enterotoxin production during the manufacturing and storage of Domiati cheese.

**Methodology:** Fresh cow's milk (9 L), locally produced on the day of the experiment, was salted (10%) and pasteurized at 63° C for 30 min. The prepared inoculum was added to the warmed milk (35-40 °C) in a count of log 6 CFU/ml. The inoculated milk was divided into the control group, milk containing a yoghurt starter culture, and milk with 35µg/ml nickel oxide nanoparticles (NiO NPs). Survival Weibull and log linear models were fitted to observed data. Statistical analyses were performed by an ANOVA with a Tukey post-hoc test ( $p < 0.05$ ).

**Results:** The obtained results showed that the number of the inoculated *S. aureus* decreased at the 28<sup>th</sup> day of ripening in the control cheese. On the other hand, the mean log count of *S. aureus* decreased one week earlier (at 21<sup>st</sup> day of storage) by using yoghurt starter culture during cheese manufacturing. SEA was identified in the control cheese and could not be detected by adding of yoghurt starter culture. Notably, Domiati cheese contained MIC of NiO NPs resulted in a significant decrease in *S. aureus* counts since at day 21<sup>st</sup> of cheese maturation it was not detected (<10 CFU/g). Survival ability was well characterized by the Weibull models ( $R^2 > 0.88$ ) showing a downward concavity shape.

**Conclusion and Relevance:** Overall, the current study indicated that the addition of yoghurt and NiO NPs during processing of Domiati cheese could be useful candidates against *S. aureus* and enterotoxin production in dairy industry. This study could be useful for cheesemakers and stakeholders to set specific formulations based on the use of novel preservation technologies to inhibit enterotoxigenic *S. aureus* growth in Domiati cheeses.

**Keywords:** Domiati cheese; *S. aureus*; SEA; starter culture; microbial inactivation

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### 83: EXTENSION OF THE MICROBIOLOGICAL SHELF-LIFE OF REFRIGERATED VACUUM-PACKED TUSCAN SAUSAGE TREATED WITH ANTIMICROBIAL OREGANO (*ORIGANUM VULGARE*) AND ROSEMARY (*ROSMARINUS OFFICINALIS*) ESSENTIAL OILS

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**Introduction:** Lactic acid bacteria (LAB) are the main bacterial group associated to the spoilage of meat products. In this study, the influence of oregano and rosemary essential oils on the microbiological shelf life of refrigerated vacuum-packed Tuscan sausage was evaluated.

**Methodology:** Two different criteria were adopted to estimate the microbiological shelf life: as the time to the LAB population reaches a level of  $10^6$  and  $10^7$  CFU/g. Both essential oils were obtained through steam distillation. To model the native LAB population, a reparameterized version of the Baranyi-Roberts growth model without lag was used. In addition to the control (without the addition of oil), sausage samples were separately treated with different concentrations of each essential oil (0.05 wt%, 0.1 wt%, 0.2 wt%, and 0.4 wt%).

**Results:** It was obtained that after the addition of lower doses (0.05 wt% and 0.1 wt%) of essential oil to the sausage, the rosemary essential oil provided a higher shelf life extension of the sausages (approximately 3 and 5 days depending on the criterion considered) in relation to the oregano (approximately 1 and 3 days, respectively). On the other hand, after the addition of higher doses (0.2 wt% and 0.4 wt%), the oregano essential oil resulted in a larger increase of the shelf life of the samples (about 8 and 14 days, respectively) in relation to rosemary essential oil (about 7 to 11 days, respectively). It was observed that all of the treatments retarded the growth of the lactic acid bacteria but did not change the maximum bacterial population. Empirical expressions that relate the microbiological shelf life of the sausage with the oil concentration were derived.

**Conclusion and Relevance:** The results obtained in this study demonstrate the effectiveness of the addition of essential oils of oregano and rosemary to extend the microbiological shelf life of vacuum-packed Tuscan sausage. After the application of low concentrations of oil that do not significantly affect the sensory properties of the sausage results seem to suggest that the addition of rosemary essential oil to the sausages could provide better results than oregano essential oil.

**Keywords:** Tuscan sausage; microbiological shelf life; oregano essential oil; rosemary essential oil; lactic acid bacteria

## 122: EFFECT OF PHYSICOCHEMICAL CHARACTERISTICS OF LAMB MEAT ON ITS MICROBIOLOGICAL DETERIORATION

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**Introduction:** A common strategy to improve the tenderness of lamb meat of autochthonous Portuguese breeds is to mature vacuum-packed (VP) meat for ~7 days. Nonetheless, the extent to which microbial spoilage is delayed by VP depends upon the meat intrinsic properties. The objective of this study was to appraise how the growth rate of deteriorating bacteria in VP lamb meat is affected by the initial microbial load and physicochemical characteristics.

**Methodology:** Fifteen four-month old animals from Bordaleira-de-Entre-Douro-e-Minho (BEDM) and 15 from Churra-Galega-Bragançana (CGB) breeds were slaughtered at the same abattoir (day zero), and after 24-hour chilling, *L. dorsi* sections were vacuum-packed for microbiological analysis of mesophiles [MES], psychrotrophic [PSY], lactic acid bacteria [LAB] and *Pseudomonas* spp. [PSE] on days 3, 9 and 15. Proximate composition (db), pH and water activity of meat were determined on day 1. For each bacterium, mixed models were adjusted to assess the effects of breed and maturation in separate interaction with every intrinsic property.

**Results:** A high meat pH (F value=0.72 for MES, F=0.97 for LAB, F=1.17 for PSE and F=0.13 for PSY) was not as determinant of higher initial microbial counts as it was of faster microbial growth (F=84.2 for MES, F=28.6 for LAB, F=20.3 for PSE and F=65.2 for PSY). Fat content (F=13.6 for MES, F=4.43 for LAB, F=2.95 for PSE and F=10.3 for PSY) also affected microbial growth rates, yet not as much as *A<sub>w</sub>* (F=82.3 for MES, F=28.1 for LAB, F=20.4 for PSE and F=63.6 for PSY) and protein content (F=80.6 for MES, F=29.4 for LAB, F=20.8 for PSE and F=63.3 for PSY). Although BEDM lamb meat presented higher initial counts ( $p<0.05$ ) than CGB meat in all bacterial groups, the effect of breed on spoilage indicator counts (F=8.3 for MES, F=14.8 for LAB, F=6.34 for PSE and F=9.90 for PSY) was not as strong as the effect of meat intrinsic properties.

**Conclusion and Relevance:** To extend current shelf-life of VP Portuguese lamb meat, it is key to implement practices that ensure a fast drop of carcass pH at slaughter, and to investigate the factors causing the higher spoilage bacteria counts associated with the BEDM breed.

Keywords: Lactic acid bacteria; psychrotrophic; *Pseudomonas*; spoilage; pH

## 123: MICROBIAL CONTAMINATION OF LAMB CARCASSES AND MEAT FROM AUTOCHTHONOUS PORTUGUESE BREEDS

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**Introduction:** Despite the high consumption of lamb meat in Portugal, there is limited information on its microbiological safety. The objective of this study was to evaluate the levels of hygiene indicator microorganisms and occurrence of pathogens on lamb carcasses and meat from two autochthonous Portuguese breeds, Bordaleira-de-Entre-Douro-e-Minho (BEDM) and Churra-Galega-Bragançana (CGB); and evaluate possible associations among these bacterial groups within slaughter batches.

**Methodology:** On 11 sampling visits, 30 BEDM and 30 CGB four-month old lambs were slaughtered, and 400-cm<sup>2</sup> pooled neck/loin/hind swabs were taken after carcass dressing. After 24-hour chilling, *L. dorsi* sections were vacuum-packed (VP) and stored at 4°C. Swab samples were analysed for mesophiles, coliforms, *Escherichia coli*, *Salmonella* spp., *L. monocytogenes* and *E. coli* O157, while meat samples were analysed for *Salmonella* on the 3<sup>rd</sup>, 9<sup>th</sup> and 15<sup>th</sup> day after slaughter. Linear and logistic mixed models were adjusted to assess any effect of breed on microbial counts/prevalence.

**Results:** BEDM lamb carcasses presented higher counts ( $p < 0.05$ ) of mesophiles (3.52 log CFU/cm<sup>2</sup>), coliforms (0.936 log CFU/cm<sup>2</sup>) and *E. coli* (0.307 log CFU/cm<sup>2</sup>) than CGB carcasses (3.03, 0.633 and 0.079 CFU/cm<sup>2</sup>, respectively), probably arising from the longest hair of BEDM sheep introducing greater contamination into the slaughter lines. In terms of pathogens, there was no difference between BEDM and CGB in the incidences of *Salmonella* spp. (21.4% [95% CI: 10.0–40.2%] versus 16.7% [7.10–34.3%]), *L. monocytogenes* (3.50% [0.50–21.4%] versus 6.70% [1.60–23.1%]) and *E. coli* O157 (32.1% [17.6–51.1%] versus 16.7% [7.10–34.3%]). On a batch basis, the presence of *E. coli* O157 was not associated with higher counts of coliforms ( $p = 0.812$ ) or *E. coli* ( $p = 0.706$ ). However, when *Salmonella* was found in a sampled batch of lamb carcasses, the odds of finding *Salmonella* in meat, at a later stage in the chain, increased by 8.705 times ( $p = 0.078$ ).

**Conclusion and Relevance:** This study revealed the potential public health risk posed by lamb meat due to the frequent contamination of their carcasses with *E. coli* O157, *Salmonella* spp. and *L. monocytogenes*, in decreasing order. Beyond the improvement in slaughter hygiene, further investigation should focus on on-farm interventions such as improved husbandry, feeding with probiotics and use of vaccines.

**Keywords:** *Listeria monocytogenes*; *Escherichia coli* O157; *Salmonella*; coliforms; abattoir

## 126: CHARACTERISING THE FATE OF *LISTERIA MONOCYTOGENES* IN ARTISANAL MINAS SEMI-HARD CHEESE DURING RIPENING

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**Introduction:** Artisanal Minas semi-hard cheese (AMC) is traditionally made with raw cow's milk employing indigenous lactic acid bacteria (LAB) and ripened for 14-22 days. The objective of this study was to characterise the fate of *Listeria monocytogenes* (LM) in AMC during ripening, as influenced by cheese pH and the presence of autochthonous or intentionally-added anti-listerial LAB.

**Methodology:** Four treatments of AMC were prepared from raw or pasteurised milk with or without addition of selected LAB with known anti-listerial activity. Cheeses were analysed for pH and LAB/LM counts throughout ripening (22±1°C). The Huang primary model and a log-linear decay model were used to model the independent LAB and LM growth/decay; the dynamic tertiary Huang-Cardinal[pH] model was used to describe LM growth, which occurred in the treatments without LAB addition; and the Jameson-effect model with the total maximum cell density  $N_{\max \text{ tot}}$  was used to model simultaneously LM and LAB in cheese during ripening.

**Results:** LM inactivation occurred in both treatments with added LAB, however cheeses produced with raw milk presented a faster inactivation rate ( $-0.0260 \ln \text{ CFU/g h}^{-1}$ ) than those produced with pasteurised milk ( $-0.0182 \ln \text{ CFU/g h}^{-1}$ ). For the treatments without LAB addition, LM growth capacity was higher when pasteurised milk was used (18.3  $\ln \text{ CFU/g}$  vs. 16.2  $\ln \text{ CFU/g}$  for raw milk). Optimum growth rates for LM in Minas cheese with indigenous LAB made of raw (0.346  $\ln \text{ CFU/g h}^{-1}$ ) and pasteurised milk (0.198  $\ln \text{ CFU/g h}^{-1}$ ) could be determined by the Huang-Cardinal[pH] model. However, the Jameson-effect with  $N_{\max \text{ tot}}$  was more flexible as it was capable of representing both the growth and death of LM, which the Huang-Cardinal[pH] cannot do. By the Jameson-effect model, it was observed that LAB presence inhibited pathogen growth in all treatments ( $P < 0.05$ ), except in that made of pasteurised milk without LAB addition (interaction parameter  $\gamma < 1$ ,  $P = 0.589$ ).

**Conclusion and Relevance:** The Jameson-effect model demonstrates a more accurate representation of microbial dynamics in AMC, and is a valuable tool to assist in future risk assessments managing the risk of listeriosis.

**Keywords:** Artisanal cheese; lactic acid bacteria; dynamic modelling; Cardinal model; Jameson-effect

### **130: PARTIAL SUBSTITUTION OF WHEAT FLOUR WITH MESQUITE FLOUR FOR TEXTURAL QUALITY AND SHELF LIFE IMPROVEMENT OF THE TRADITIONAL BRAGANÇA BREAD**

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**Introduction:** The artisanal Bragança bread is a highly appreciated product in the region, although it stales very fast. The objective of this study was to evaluate the effect of partial substitution of wheat flour with mesquite (*Prosopis pallida*) flour on the textural properties and shelf-life of the bread.

**Methodology:** The dough was prepared with different substitution levels of mesquite flour (0.0, 5.0, 10.0 and 15.0%) and wheat flour type (65 and 55), correcting every formulation for water absorption. Specific loaf volume, baking loss, texture profile analysis [TPA] features, water activity and digital image analysis features were analysed 24 h after baking, while for shelf-life analysis, TPA hardness and water activity [aw] were measured on days 1, 3 and 5.

**Results:** Formulations with mesquite flour presented loaves with higher ( $p<0.001$ ) specific volume, baking loss, mean cell density and cell size uniformity; and lower ( $p<0.001$ ) crumb aw, hardness, cohesiveness, chewiness, resilience, mean cell area and void fraction. A higher mesquite dose showed to have no effect on the mean compactness and mean aspect ratio of the crumb alveoli. Compared to wheat flour type 65, wheat flour type 55 yielded loaves with higher ( $p<0.001$ ) loaf specific volume and baking loss, bread crumb springiness and mean cell area; and lower ( $p < 0.001$ ), bread crumb hardness, cohesiveness, chewiness, mean cell density, cell size uniformity and cell compactness. Wheat flour type 55 showed to have no effect on bread crumb aw and resilience.

For both flours 65 and 55, the higher the mesquite dose, the lower the initial resilience and the lower the initial aw of the bread crumb. Whereas the substitution of flour 65 with mesquite flour did not delay the rate of bread staling, the use of flour 55 produced an increase in hardening rate ( $p=0.028$ ), but a decrease in loss of resilience ( $p=0.009$ ) and in dehydration rate ( $p<0.001$ ).

**Conclusion and Relevance:** Softness, elasticity, chewiness and crumb porosity of Bragança bread can be improved by substituting 5-10% wheat flour type 55 with mesquite flour. Addition of mesquite flour delays dehydration and loss of resilience of bread crumb only when using wheat flour type 55.

**Keywords:** Mesquite flour; wheat flour; fibre enrichment; texture profile analysis; shelf-life analysis

### 131: EVOLUTION OF LAMB MEAT QUALITY TRAITS UNDER PROLONGED VACUUM STORAGE

Gisela Rodrigues<sup>1</sup>, Jose Lorenzo<sup>2</sup>, Ursula Gonzales-Barron<sup>1</sup>, Vasco Cadavez<sup>1</sup>

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**Introduction:** Meat quality, as judged by the consumer, is mainly dictated by colour, juiciness and tenderness. Wet maturation of meat done by vacuum-packaging (VP) allows the ageing process to continue, yet in conditions that retard meat spoilage. The objective of this study was to evaluate the evolution of meat quality traits (colour, TBARs, water retention capacity [WRC] and Warner-Bratzler shear-force [SF]), and their interrelationships with physicochemical characteristics (pH, water activity [aw] and proximate composition) in VP lamb meat during refrigeration.

**Methodology:** Fifteen four-month old lambs from Bordaleira-de-Entre-Douro-e-Minho (BEDM) and 15 from Churra-Galega-Bragançana (CGB) breeds were slaughtered in an abattoir (Day 0). The ultimate pH [pH<sub>24</sub>] was measured 24 h after slaughter; and *Longissimus dorsi* muscle was cut in three segments, vacuum-packed and stored at 4°C. On day 1, proximate analysis was determined. On days 3, 9 and 15, pH, aw, Hunter L\*, a\*, b\*, WRC, TBARs and SF were measured. For each meat quality trait, mixed models were adjusted to assess the effects of breed, sex, maturation time and physicochemical properties.

**Results:** All quality traits were affected by maturation time ( $p < 0.001$ ), excepting L\* ( $p = 0.144$ ). There was no effect of sex on meat quality traits, while breed had an effect on colour, with CGB breed producing meats of lower L\* ( $p = 0.050$ ), a\* ( $p = 0.004$ ) and b\* ( $p = 0.005$ ). While BEDM meats were more tender ( $p = 0.002$ ) than CGB meats, breed was not found to affect either TBARs ( $p = 0.408$ ) or WRC ( $p = 0.680$ ). None of the physicochemical properties modulated WRC. Meats of lower aw presented lower L\* ( $p = 0.065$ ), a\* ( $p = 0.072$ ) and SF ( $p = 0.001$ ), and higher TBARs ( $p = 0.051$ ). Fat content was found to inversely affect only SF ( $p = 0.014$ ) while protein content did not modulate any quality trait. pH<sub>24</sub> had a strong effect ( $p < 0.001$ ) on b\* and SF. Although meat became more tender from day 3 to 9, it was not significantly improved from day 9 to 15 (final SF =  $2.96 \pm 0.155$  kg/cm<sup>2</sup>).

**Conclusion and Relevance:** The evolution of colour and tenderness of VP lamb meat was different between breeds. Hence, further studies should be undertaken to elucidate the effect of breed disentangling it from that of pH.

**Keywords:** Colour; water retention capacity; shear-force; TBARs; maturation

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## 145: INVESTIGATION OF LAMB AS A SOURCE OF SHIGATOXIN-PRODUCING *ESCHERICHIA COLI* IN PORTUGAL

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**Introduction:** The gastrointestinal tract of ruminants constitutes the main natural reservoir of shigatoxin-producing *Escherichia coli* (STEC), which is a serious pathogen that can be transmitted to humans through contaminated raw/undercooked meat products, raw milk and raw vegetables. However, the number of epidemiological studies investigating Portuguese sheep as a source of STEC strains is quite limited. Thus, the objective of this work was to assess the occurrence of STEC in apparently-healthy lambs from Portuguese breeds – Churra-Galega-Bragançana (CGB) and Bordaleira-Entre-Douro-e-Minho (BEDM), and determine the presence of major virulence genes in the isolates.

**Methodology:** Six sheep farms located in Northeastern Portugal were visited, and recto-anal samples from a total of 50 CGB and 7 BEDM 4-month old lambs were taken, and enriched in 9-mL TSB with 20 mg/L novobiocin. After incubation at 37°C for 24 h, samples were streaked onto SMAC agar with 0.05 mg/L cefixime and 2.5 mg/L potassium tellurite, and incubated at 37°C for 24 h. Typical colonies were confirmed biochemically by indole, methyl-red and Voges-Proskauer tests, and serologically by O157 latex agglutination. Purified isolates were subjected to multiplex PCR to determine the genes encoding for shigatoxin (*stx1*, *stx2*), enterohemolysin (*hlyA*), and intimin (*eae*).

**Results:** From the BEDM breed samples, no *E. coli* O157 was recovered (0/7), while only two shedders of sorbitol-negative *E. coli* were found in the CGB group (incidence 4.0%; 95% CI: 1.1 – 13.5%). PCR analysis of 10 isolates of sorbitol-negative *E. coli* coming from one positive lamb revealed the presence of two different strains, one coding for *stx1* gene (8/10=80%) and the other coding for both *stx1* and *hlyA* genes (3/10=30%). Sorbitol-negative colonies (3) isolated from one animal did not present any of the genes.

**Conclusion and Relevance:** Although the overall prevalence of shigatoxin-producing *E. coli* in recto-anal contents from apparently-healthy lamb was low (1.75%; 95% CI: 0.09 – 10.63%), these findings are important for public health. Preventive measures are necessary to control the incidence of STEC infections in sheep and people. Good hygiene practices are necessary particularly in the slaughterhouse where contamination of lamb meat and environment with intestinal contents can be prevented.

**Keywords:** Sheep; recto-anal swab; PCR; virulence



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**147: PREVALENCE AND GENOTYPE IDENTIFICATION OF *SALMONELLA* spp. ISOLATED FROM A MEAT PRODUCT ARTISANALLY PRODUCED IN BRAGANÇA**

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**Introduction:** Previous meta-analytical work estimated that in Portuguese meat products intended to be eaten cooked, the overall occurrence of *Salmonella* spp. was 9.7% (95% CI: 7.0–13.4%). One of these meat products is alheira, which is a fermented sausage made of poultry/pork meat, bread and seasonings. The objective of this study was to investigate prevalence, numbers and serovars of *Salmonella* spp. in alheira sausages artisanally produced in Bragança, Portugal.

**Methodology:** This work was undertaken in three stages: (i) sampling of 52 alheiras from markets and traditional fairs; (ii) detection of *Salmonella* using culture methods and enumeration by MPN; and (iii) molecular confirmation of isolates (*invA* and randomly-cloned chromosomal fragment), and typing of *S. Enteritidis* (*SefA*), *Typhimurium* (*fliC*) or *Pullorum* (*glgC*) by PCR.

**Results:** Analysis of 52 sausage samples revealed the presence of *Salmonella* spp. in 8 samples (incidence of 0.154; 95% CI: 0.080–0.275), although all of these positive samples were unpacked sausages from traditional fairs (n=21), indicating therefore the higher *Salmonella* prevalence in alheiras sold in these establishments (incidence 0.381; 95% CI: 0.207–0.591). *Salmonella* mean concentration was 1.938 log MPN/g (s.d 0.839 log MPN/g). All of the 33 biochemically- and serologically-confirmed isolates coded for the *invA* gene. Multiplex-PCR revealed that only 3 of the positive isolates had the presence of *SefA* genes; which indicated that 9.1% of the isolates belonged to *Enteritidis*; while 20 isolates belonged to *Typhimurium* (60.6%) since they coded for *fliC* gene. The other 10 isolates (30.3%) were of serovars different from *Enteritidis*/*Typhimurium*/*Pullorum* since they only presented genes general for *Salmonella*. From the positive alheiras, *Enteritidis* or *Typhimurium* serovar was recovered from two samples, while the other samples harboured at least two serovars.

**Conclusion and Relevance:** *Salmonella* continues to be a frequent contaminant of alheiras produced in Bragança, and, in particular, of those sold in local fairs (38%). Not unexpectedly, *Typhimurium* and *Enteritidis* represented the prevailing serovars (72.7%) since they are linked to pork and poultry meat, the main raw materials of alheira. Regional producers must be urgently informed on the implementation of preventive and corrective actions in their current manufacturing processes and hygiene standards.

**Keywords:** Alheira; Portugal; PCR; molecular typing

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## 148: OPTIMISATION OF CMC, XANTHAN AND GUAR GUMS AS GLUTEN REPLACERS FOR THE ELABORATION OF QUINOA-BASED BREAD

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**Introduction:** Several investigations have been dedicated to the development of gluten-free (GF) bread because baked goods lacking a gluten matrix have poor technological quality. The study of hydrocolloids combinations in food products can be done through the use of mixture design experiments, where the quality responses measured are assumed to depend only on the relative proportions of the mixture components. Thus, the objectives of this study was to investigate, by means of a D-optimal mixture design, the combined effects of carboxymethyl cellulose (CMC), xanthan (XG) and guar (GG) gums on quality properties of quinoa-based GF bread.

**Methodology:** GF breads were elaborated with: rice (50%), corn (30%) and quinoa (20%) flours, sunflower oil (6.0%), sugar (3.0%), salt (1.5%) and dry yeast (3.0%). The ten formulations, obtained from the experimental design, used 115% of water and a total of 5% hydrocolloids consisting of mixtures of CMC (3.50 – 4.50%), GG (0.25 – 0.50%) and XG (0.25 – 1.00%). A series of physicochemical, gravimetric, rheological and bread crumb image analyses were carried out for every formulation. However, the doses of CMC, XG and GG were optimised with basis only on three consumer-driven quality attributes of bread: specific volume (SVO), texture profile analysis' bread crumb hardness (HARbr) and image analysis' mean cell area (MCA) using the Design Expert software.

**Results:** Highest SVO was obtained with a combination of a high-intermediate CMC dose (4.37%), a low-intermediate GG dose (0.38%) and a low XG dose (0.25%). Lower HARbr (i.e., softer crumbs) and higher MCA (i.e., more open grain texture) were obtained with a combination of a high-intermediate CMC dose (4.40%), a low XG dose (0.25%) and an intermediate GG dose (0.35%).

**Conclusion and Relevance:** Higher CMC or GG doses and lower XG dose produced bread loaves of greater volume, while smaller GG or XG doses produced softer bread crumbs of more open porosity. The optimum hydrocolloid mixture was obtained with 0.25% XG, 0.37% GG and 4.38% CMC, which was validated in a separate baking trial. A bread formulation that adds values to quinoa, one of the most nutritious Andean grains, has been developed by optimising widely-used hydrocolloids as gluten-replacers.

**Keywords:** Quinoa flour; texture profile analysis; mixture design; image analysis

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## Poster Session 3: Advances in Software and Databases Tools; and Advances in Risk Assessment Methods and Integration of Omics Techniques

Time: 10:40 - 11:10

Date: 19th September 2019

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### 23: FSK2R: A NEW R LIBRARY TO SUPPORT FOOD SAFETY KNOWLEDGE MARKUP LANGUAGE (FSK-ML)

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**Introduction:** Predictive models have gained popularity during the last decades in the food safety context, becoming also a fundamental component of Quantitative Risk Assessments. Until recently, due to the lack of a standardized model exchange language, scientists had to re-implement models already published in the scientific literature. Within the RAKIP initiative, the “Food Safety Knowledge Markup Language” (FSK-ML) was developed providing an information exchange language, facilitating the distribution of mathematical models in the field.

**Methodology:** Functions for creating, importing, editing, querying and exporting FSK-ML compliant files have been implemented in the R programming language. They have been compiled as an R package according to CRAN guidelines and uploaded to this repository. The package is fully documented, including function documentation and a vignette (a user manual). Furthermore, a user-friendly web application has been developed using the *shiny* R package.

**Results:** The R package FSK2R has been developed and uploaded to CRAN, making it publically available. It includes functions for importing existing models compliant with FSK-ML into R, where they can be edited and queried. It also enables running the model simulations, as well as defining new ones. Furthermore, it includes an assistant to convert existing R models into FSK-ML compliant files. This concept was demonstrated through integration of FSK2R and accompanying *shiny* application modules into the existing Bioinactivation portal, that now offers its users to export new models as a FSK-ML compliant model file.

**Conclusion and Relevance:** The FSK2R package includes all necessary functions to ease the adoption of the FSK-ML standard for mathematical models developed in R. It also supports the import of existing FSK-ML R models and their metadata. Due to the popularity of the R programming language, FSK2R can facilitate the broad adoption of

this information exchange format within the food safety community. As demonstrated via the integration of FSK2R into the existing Bioinactivation portal this new R library can serve as a bridge between existing community modelling resources and the new information exchange format FSK-ML. Therefore, this work supports harmonized and more efficient knowledge exchange in the food science community.

Keywords: FSK-ML; QMRA; information exchange; modelling; R software

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### **43: THE ONE HEALTH SURVEILLANCE CODEX – A HIGH-LEVEL FRAMEWORK TO FACILITATE EFFICIENT INFORMATION EXCHANGE ACROSS ONE HEALTH SECTORS**

Tasja Buschhardt<sup>1</sup>, Taras Guenther<sup>1</sup>, Fernanda Dorea<sup>2</sup>, Matthias Filter<sup>1</sup>

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**Introduction:** Predictive models are an essential part of risk assessment and management, which play an important role in combating zoonotic diseases and antimicrobial resistance (AMR). To provide holistic risk assessments a cross-sectorial One Health (OH) thinking is needed that supports integration of data from the animal, food, human and environmental interface into models. However, cross-sectorial information integration is still challenging as there is no true cross-sectorial, high-level framework guiding domain experts towards harmonized information exchange. The One Health Surveillance (OHS) Codex aims at providing such a framework.

**Methodology:** The OHS Codex consist of two main components 1) a set of high-level principles that should be followed and 2) a description of technical solutions and example implementations supporting the adoption of each OHS Codex principle.

**Results:** The first version of the OHS Codex postulates the following four guiding principles: 1) Support knowledge exchange; 2) Support cross-sectorial communication; 3) Support report harmonization; and 4) Support data interoperability. The proposed technical solutions for these principles are: 1) a web-based OHS Knowledge Hub; 2) a cross-sectorial OH glossary; 3) a Consensus Report Annotation Schema (CRAS); and 4) the adoption of the Linked Open Data (LOD) paradigm.

**Conclusion and Relevance:** The OHS Codex is a community effort to improve information exchange and mutual understanding driven by experts from the animal health, food and public health sectors. The generality of the OHS Codex will promote adoption in these domains and beyond. The predictive modelling and risk assessment community will thereby be supported through improved availability, findability and exchange of cross-sectorial information (e.g. models, data, parameter estimates, knowledge etc.) from the animal, food, human and environmental sectors. Experiences from pilot studies, testing the adoption of the OHS Codex, will feed back into the OHS Codex design. The OHS Codex provides the first consistent guidance to all OH related disciplines on measures to improve cross-sectorial information exchange and integrated data analysis to fight foodborne zoonotic diseases and AMR.

Keywords: One Health; harmonisation; information exchange guidance; framework; glossary

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## 65: DEVELOPMENT AND IMPLEMENTATION OF POLYNOMIAL AND GAMMA MODELS FOR LISTERIA GROWTH IN ROAST BEEF IN THE NEW LISTWARE TOOL

Taran Skjerdal<sup>1</sup>, Lars Erik Gangsei<sup>2</sup>, Ole Alvseike<sup>2</sup>, Mariem Ellouze<sup>3</sup>, Anja Kristoffersen<sup>1</sup>, Lena Haugland Moen<sup>1</sup>, Ane Osland Mohr<sup>1</sup>, Sigrun Hauge<sup>2</sup>

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**Introduction:** The risk management of *Listeria monocytogenes* in ready-to-eat foods (RTE) involve large expenses and time spent for the food business operators (FBO). The objective of this study was to develop growth models for roast beef and to incorporate them into a user-friendly risk management software tool, which is currently being developed by Norwegian meat industry in collaboration with researchers.

**Methodology:** An experimental design was prepared with 51 combinations; storage temperature (4-12°C), added acetate (0-1000 ppm), added lactate (0-4000 ppm), max core temperature of the beef (48-63°C), in addition to 3 package methods; air, vacuum, and modified atmosphere (MAP with 50 % CO<sub>2</sub>). Middle points were included. A mix of *Listeria monocytogenes* strains were inoculated on slices of roast beef before packaging and incubation. For each factor combination, the *Listeria* concentrations were analysed 8-12 times during storage. Measurements of pH, aw, lactic acid bacteria, total plate count, lactate and acetate were performed. Two models were developed; a polynomial model based on linear regression of the four factors in the study, and a gamma model with gamma functions for temperature, pH, a<sub>w</sub> based on reported cardinal values for growth and MIC values for acetate and lactate.

**Results:** The storage temperature was the most important factor for growth. The results showed a substantial inhibiting effect of acetate, but less for lactate. MAP packaging gave lower growth than vacuum and air package. The core temperature of the meat did not affect the growth. Both models were implemented in the web-based software. Predicted growth with 10, 50 and 90 % likelihood of exceeding a 2 log increase during storage was displayed. The two models predicted similar growth rates. Validation studies with new samples indicated correct estimation or overestimation of the time until a 2 log increase. Most deviation was observed at low storage temperature where acetate was added to the roast beef.

**Conclusion and Relevance:** The validity of the models will be further tested with roast beef and similar products from other countries, in order to investigate the validity of the models for other meat products with similar characteristics as roast beef.

Keywords: *Listeria monocytogenes*; ready-to-eat food; polynomial model; gamma model; decision support tool

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## **67: ENABLING EFFICIENT FOOD SAFETY KNOWLEDGE EXCHANGE WITH THE OPEN SOURCE SOFTWARE FSK-LAB**

Ahmad Swaid, Miguel de Alba, Carolina Plaza-Rodriguez, Tasja Buschhardt, Lars Valentin, Octavio Mesa-Varona, Taras Günther, [Matthias Filter](#)

Federal Institute for Risk Assessment, Berlin, Germany

**Introduction:** In the last decades, a large number of models have been developed in the quantitative microbial risk assessment and predictive microbiology domains with different scripting languages (e.g. R, Matlab) and tools (e.g. @Risk, FDA-iRISK). However, the exchange and combination of such models originating from different platforms is still a great challenge. Recently, an information exchange format called Food Safety Knowledge Markup Language (FSK-ML) has been developed that defines a framework for encoding all relevant data, metadata and model scripts in a harmonized machine-readable format. Food Safety Knowledge Lab (FSK-Lab) is a user-friendly software tool that can create, read, write, execute and combine FSK-ML compliant models.

**Methodology:** FSK-Lab extends the open source Konstanz Information Miner (KNIME) software, which is a graphical programming tool allowing users to create data analysis workflows from building blocks (nodes). FSK-Lab nodes were programmed in Java as Eclipse plugins.

**Results:** FSK-Lab is a set of modular, open-source KNIME nodes for the generation, annotation and execution of script based food safety models. FSK-Lab provides also functionalities to combine or modify existing models via a graphical user interface. The workflow-based software architecture supports modellers in their efforts to provide full transparency and reproducibility, as the complete model generation and model execution workflow can be shared including all the intermediate results.

**Conclusion and Relevance:** FSK-Lab can be characterized as a generic graphical modelling toolbox that also supports the exchange and integration of existing risk assessment models from different programming languages, as e.g. R, Java, Python. An essential aim of FSK-Lab is the support (reading and writing) for the information exchange format FSK-ML and the ability to combine existing models generated in different programming languages. FSK-Lab is the first tool that fully supports the information exchange format FSK-ML. FSK-ML is currently the only information exchange format in the food safety domain that allows describing food safety models generated in different programming languages in a harmonized way. FSK-Lab also supports modellers in their efforts to provide complete metadata for their models and to adopt the proposed FSK-ML format.

**Keywords:** FSK-ML; QMRA; information exchange; open source software; R software

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## 68: RISK ASSESSMENT MODELLING AND KNOWLEDGE INTEGRATION PLATFORM (RAKIP)

Virginie Desvignes<sup>1</sup>, Lars Valentin<sup>2</sup>, Laurent Guillier<sup>1</sup>, Moez Sanaa<sup>1</sup>, Maarten Nauta<sup>3</sup>, Matthias Filter<sup>2</sup>

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**Introduction:** In risk assessment domain, major efforts have been undertaken to provide a large variety of models and data. However, there is a lack of solutions that enables efficient dissemination and exploitation of existing knowledge to the whole food safety community. The Risk Assessment Modelling and Knowledge Integration Platform (RAKIP) project was created as a joint ANSES, BfR and DTU Food initiative aiming at making available open resources that facilitate the exchange of food safety knowledge in a transparent and consistent way.

**Methodology:** To achieve efficient knowledge exchange there is the need to develop harmonized information around the models and data. Four critical steps are related to the development of a harmonized conceptual description of data and modelling activities in the domain, the development of generic metadata schema including controlled vocabularies, the development of rules for knowledge annotation and the development of an open information exchange format (FSK-ML) and web-based repository.

**Results:** Proper definition of annotation metadata concepts is a prerequisite for correct understanding of models or data sets. For this, the RAKIP community defined a structured metadata schema (<https://goo.gl/PE4ysP>), which describes relevant concepts for annotating data or models in the risk assessment in food as the type of hazard, matrix, sampling plan, distribution of parameters of a model etc. Additional information is provided regarding the mandatory status of the metadata concept and if there is a controlled vocabulary associated to the metadata. Controlled vocabulary allows the annotation harmonization and is mainly based on the terms from existing ontologies, exchange formats and tools (SSD2, PMM-Lab). For completing some metadata, free text can be preferred. The list of the controlled vocabulary is available at <https://goo.gl/wbFoZU>.

**Conclusion and Relevance:** The RAKIP Model Repository is a web-based repository for data and models that can be freely assessed by any user, from risk assessor to modeller. It currently contains mainly risk assessment and predictive microbiology models provided by modellers of RAKIP partners in the FSK-ML format. It is also possible to execute simulations online with user-defined parameters for those models available within the repository (<https://foodrisklabs.bfr.bund.de/rakip-model-repository-web-services>).

**Keywords:** Risk assessment; knowledge sharing; data annotation; metadata; harmonised vocabulary

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## 70: MIRARAM: MINIMUM INFORMATION REQUIRED TO ANNOTATE A FOOD SAFETY RISK ASSESSMENT MODEL

Estibaliz Lopez de Abechuco<sup>1</sup>, Octavio Mesa Varona<sup>1</sup>, Tasja Buschhardt<sup>1</sup>, Marios Georgiadis<sup>2</sup>, Matthias Filter<sup>1</sup>

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**Introduction:** In the last decades a large number of mathematical models have been developed to support food safety risk assessments of microbiological and chemical hazards. However, the exploitation and re-use of this knowledge by the risk assessment community is hampered due to the heterogeneity of software tools used to create the models and the lack of a standardized information exchange format to efficiently share them. The implementation of annotation guidelines and information exchange formats for risk assessment models would greatly facilitate the knowledge exchange and, therefore, the re-use and further improvement of models.

**Methodology:** Here we propose a guideline focused mainly on food safety risk assessment models, called MIRARAM (Minimum Information Required to Annotate a food safety Risk Assessment Model), which describes how and what minimal metadata should be provided for the annotation of a mathematical model.

**Results:** The MIRARAM guideline is divided into two main parts. Firstly, the general guiding principles are postulated; these principles were adapted from best practice minimum information guidelines previously established in other scientific disciplines. Secondly, a minimal set of metadata and a specific guidance on technical requirements are provided to enable the fulfilment of the general guiding principles. The minimal set of model metadata was selected from a domain-specific community resource called “Metadata Master Schema” that has been developed and described by food safety risk assessment experts before. Each metadata concept in MIRARAM is defined together with its format and, if applicable, an extendable controlled vocabulary. To avoid future format incompatibility issues, MIRARAM requests that model and model annotations should be provided in files compliant to the COMBINE (Computational Modelling in Biology Network) archive specification.

**Conclusions and Relevance:** We believe that the MIRARAM guidelines could support scientific journal editors / publishers, modellers, risk assessors and software developers in their efforts to make their valuable work reusable. Further, MIRARAM could also contribute to improve quality and transparency of the model generation process and model-based predictions. It would also help to avoid issues related to intellectual property rights, misinterpretation and potential misuse of data or models.

**Keywords:** QMRA modelling; metadata; annotation guidelines; information exchange



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## 82: fAUTHENT – AN OPEN SOURCE FRAMEWORK FOR DISTRIBUTED FOOD AUTHENTICITY DATA AND KNOWLEDGE MANAGEMENT

Lars Valentin<sup>1</sup>, Martin Horn<sup>2</sup>, Sven Böckelmann<sup>3</sup>, Tim Bartram<sup>4</sup>, Ralph Tröger<sup>4</sup>, Susanne Esslinger<sup>1</sup>, Matthias Filter<sup>1</sup>, Thomas Hirsch<sup>3</sup>

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**Introduction:** Food fraud is a significant issue in the food-sector as it is difficult to verify if a product fully complies with what the label states. For example, olive oil might be mixed with low quality oils or not originate from the declared region. While adulteration might not affect the customers health, there is the possibility that substances are added which are potentially health-threatening.

**Methodology:** NMR spectroscopy is a widely used method to analyse food authenticity. In non-targeted authentication, each measurement creates a so called (spectral) fingerprint which is unique for the tested product. This uniqueness allows to distinguish if a product is authentic or not, by comparing patterns within these fingerprints. Using a set of fingerprints from authentic food products allows to create predictive models that can classify new fingerprints (model-input) as authentic or not for a given product property (model-output). The fAuthent software framework has been developed to facilitate the sharing and exchange of such fingerprints and related product master data in a cloud-based distributed environment between all members of the supply chain including official control. All fAuthent software components are available as open source software.

**Results:** fAuthent allows food business operators, authorities, labs and researchers to access information on product metadata, analytical fingerprints and predictive models from other fAuthent-partners under the condition that access is granted by the data owner. With the fAuthent-framework an important limitation of untargeted analytical methods is addressed, as these rely currently on a central database of fingerprints. To solve this, the fAuthent framework is based on a so-called “Event repository”. Events are information-objects structured according to the industry-standard EPCIS holding information on any action taken by a fAuthent-partner related to food-authenticity, e.g. measuring a fingerprint, the provisioning of a predictive model or applying such model on a specific sample. Through the “Event repository” each fAuthent-partner keeps full control on their own data while in parallel all other stakeholders can be informed.

**Conclusion and Relevance:** The fAuthent-framework demonstrator has been designed and tested within the German FoodAuthent project. Now, additional efforts will be taken to develop this framework into a fully operational system.

**Keywords:** Food authenticity; NMR spectroscopy; predictive modelling; software framework; metadata

## **100: DEVELOPMENT OF AN ON-LINE COLLABORATIVE FRAMEWORK FOR APPLYING AND SHARING PREDICTIVE MICROBIOLOGY AND QUANTITATIVE MICROBIAL RISK ASSESSMENT MODELS**

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**Introduction:** Microhibro is an on-line software tool for predictive microbiology and Quantitative Microbial Risk Assessment (QMRA), that has shown a great potential as educational and scientific resource over the last few years since its creation in 2012. Nowadays, it is widely recognized, especially, from some transnational initiatives, that the improvement in model interpretability and output applicability is a crucial aspect in order to expand predictive model application and create a strong predictive microbiology community. In this work, the software MicroHibro was brought to a new predictive software generation, conferring new functionalities able to support a collaborative work and improve model application.

**Methodology:** The project is ambitious, from the perspective that the overall goal could only be achieved through a state-of-art and comprehensive knowledge of predictive microbiology. Therefore, MicroHibro database was restructured by setting a harmonised vocabulary to define models, food matrices, units and parameters. Model and simulation approaches implemented in MicroHibro were integrated in a hierarchical scheme, based on mathematical functions, databases and ontologies, connected by customised APIs. Thus, model definition schemes can be updated in real time, as new data are inputted in the system by curators or community contributors.

**Results:** The result is a user-friendly interface for the predictive microbiology community to define predictive model based on a customised system for mathematical model definition and model sharing and exchange. Different predictive models can be defined in MicroHibro, including survival, inactivation, growth and transfer models. Besides, dose-response models are included as these are used in the QMRA module. As result of the latest MicroHibro developments, a QMRA comparison module was incorporated, enabling to develop different QMRA scenarios or compare different products, pathogens or modelling approaches.

**Conclusion and Relevance:** New developments in MicroHibro demonstrate that predictive microbiology can move forward a collaborative and co-working approach underpinned by suitable software tools and languages (e.g. FSK-ML), providing with valuable instruments to end-users and the predictive microbiology community to develop and apply models for more reliable risk management decisions. The predictive microbiology software tools, like MicroHibro, are effective instruments to bring models to scientific community, as a harmonised and structured implemented scheme.

**Keywords:** Software tool; microbial risk management; database; microbial food safety; predictive networking

### 3: RAW DRINKING MILK: WHEN POTENTIAL HEALTH BENEFITS FACE THE FOODBORNE PATHOGENS – A RISK-BENEFIT STUDY

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**Introduction:** Milk is a highly nutritious food, and currently there is considerable debate on the potential health risks and benefits of consumption of raw milk (RM) compared to pasteurized milk (PM). Claimed health benefits are e.g. “higher nutritional value”, especially regarding vitamins, “beneficial microflora” as probiotic bacteria, and “allergy prevention”. However, several pathogens can be present in RM and have been identified as the cause of several foodborne outbreaks.

**Methodology:** The objective of this study was to assess the risk-benefit balance and quantify the health impact of RM consumption in terms of Disability-adjusted life years (DALY). The microbiological hazards *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni* and Shiga toxin-producing *Escherichia coli* were considered next to potentially beneficial components such as vitamins A and B2. Mathematical models, including predictive models of bacterial inactivation and growth, were used to quantify the DALYs associated to the consumption of RM directly from vending machines. Published data were used to perform exposure assessment (initial concentration and prevalence of pathogens, growth models of pathogens in milk according to different storage conditions), hazard characterization (dose-response models) and risk characterization (estimating the number of cases due to milk consumption). The BCoDE tool was used to estimate the associated DALYs. The Dutch food composition database was used to estimate the intake of vitamins A and B2 through milk consumption and the GBD Results Tool was used to establish the associated risk prevention and to estimate the associated DALYs.

**Results:** Although the evidence for health benefits is limited and the included pathogens do not reach high levels under proper storage, for example in extreme conditions (3 days under 8°C), 7.6 DALYs/year were estimated to be lost due to listeriosis. In contrast, preventable DALYs/year associated to vitamins would not exceed 1 DALY/year.

**Conclusions and Relevance:** Quantification into DALYs will aid the public debate on the possible benefits and risks of the growing popularity of RM consumption. The added value of such a quantitative assessment is that consumers can be informed more precisely on expected health impact of this consumption.

**Keywords:** Raw milk; risk-benefit assessment; DALY; predictive microbiology; health impact

## **52: USING NEXT GENERATION SEQUENCING TO TRACK THE OCCURRENCE OF CRONOBACTER IN A DAIRY POWDER INGREDIENT PRODUCTION FACILITY**

Qicheng Hao, Francis Butler, Friedrich von Westerholt

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**Introduction:** *Cronobacter Sakazakii* can be commonly found in the environment of dairy processing facilities and is potentially of concern due to its ability to cause illness in neonates. While *Cronobacter sakazakii* has commonly been associated with powder infant formula manufacture, attention has recently been focused on other dairy powders which potentially can be used in infant formula manufacture.

**Methodology:** A detailed surveillance study was carried out in a dairy powder production facility. Twenty environmental sampling locations were selected and were sampled monthly for 12 months. Sampling locations included equipment surfaces, walls, floors, air inlets and other surfaces such as door handles frequently touched by operators. Detection for *Cronobacter* spp was carried out according to ISO22964. All positive samples were whole genome sequenced on an Illumina Hiseq.

**Results:** Overall, the occurrence of *Cronobacter* was at its lowest in summer compared to the other times of the year. MLST profiling of positive samples indicated 4 sequence types. The Centre for Genomic Epidemiology CSI Phylogeny tool was used for SNP analysis. Phylogenetic analysis indicated 8 separate genotypes. Within each genotype, the SNP differences was very small, indicating that the isolates were essentially clonal and most likely came from the one original contamination source. Combining the phylogenetic data with the other meta data including location and sample date allowed the identification of a location within the process environment that was highly likely to have been the location of the contamination. Phylogenetic analysis also demonstrated the persistence of some strains over the year long period of the surveillance.

**Conclusion and Relevance:** NGS allows very precise ‘fingerprinting’ of pathogens isolated in food process facilities and allows advance root cause analysis to be carried out to control and manage pathogens in the process facility environment. The sequence can also underpin the risk assessment of *Cronobacter* in dairy ingredients.

**Keywords:** Next Generation Sequencing; *Cronobacter*; dairy powder; surveillance; genotype

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## 54: APPLICATION OF THE MINION SEQUENCER IN RAPID IN-DEPTH CHARACTERISATION OF *CRONOBACTER SAKAZAKII* ISOLATED FROM DAIRY BASE POWDER PROCESSING ENVIRONMENT

Yu Cao, Daniel Hurley, Qicheng Hao, Francis Butler

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**Introduction:** *Cronobacter sakazakii* is a major pathogen of concern during powdered infant formula (PIF) production. It is highly resistant to a low moisture environment, and ingestion of *C. sakazakii* contaminated PIF leads to a high death rate in neonates and infants. Potential *C. sakazakii* contamination sources in PIF include raw materials (base powder and other ingredients) and factory production environment, so it's very important to ensure their microbial qualities. A one-year environment sampling was carried out to monitor for the presence and distribution of *C. sakazakii* in the production environment of a dairy ingredient factory in Ireland, and Illumina sequencing was carried out for all the isolates identified from the factory. Multilocus sequence typing analysis indicated that the isolated *C. sakazakii* strains belonged to 4 sequence types: ST1, ST4, ST41 and ST42. For each sequence type, MinION sequencing was carried out for one isolate to acquire a complete reference genome for downstream studies and to study the bacteria genome in detail.

**Methodology:** *C. sakazakii* isolate CFS3141, sequence type ST41, was used for the current study. Genomic DNA was extracted from the isolate using Promega kit, and MinION sequencing was carried out. Basecalled MinION data was assembled *de novo* with canu.

**Results:** The complete genome, including one chromosome and two plasmid sequences, of *C. sakazakii* strain CFS3141 were generated and used as a reference for the analysis of other *C. sakazakii* strains. Presence of virulence genes, heat and sanitizer tolerance genes in the strain genome was also studied.

**Conclusion and Relevance:** Use of the MinION data allowed rapid full closure of the *C. sakazakii* sequence which was not possible using the Illumina data alone. The availability of a fully closed genome allowed subsequent phylogenetic comparison of the other ST41 isolates recovered from the process facility. This study demonstrated the advantages of using the MinION sequencing technique in association with the Illumina sequencing technique to phylogenetically characterize the *Cronobacter* isolates detected from a dairy process facility, which could ultimately contribute to the build-up of the monitoring system on the presence of *Cronobacter* in the dairy production environment.

**Keywords:** *Cronobacter sakazakii*; base powder factory; dairy production environment; whole genome sequencing; MinION sequencing

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## 57: CRITICAL ANALYSIS OF BEEF QUANTITATIVE MICROBIAL RISK ASSESSMENT MODELS

Vincent Tesson<sup>1</sup>, Michel Federighi<sup>1</sup>, Enda Cummins<sup>2</sup>, Juliana de Oliveira Mota<sup>1</sup>, Sandrine Guillou<sup>1</sup>, Géraldine Boué<sup>1</sup>

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**Introduction:** Each year in Europe, meat is linked with 10% of around 23 million annual foodborne illnesses, with a high contribution from beef meat. These illnesses are due to pathogenic bacterial contamination along the farm-to-fork chain. To prevent these illnesses, decision-making processes in food safety rely on Quantitative Microbiological Risk Assessment (QMRA). Within this context, and with several meat safety crises, the development of QMRAs has expanded in recent decades. This study aimed to perform a critical analysis of existing QMRAs of beef meat and assess future challenges.

**Methodology:** A critical analysis of beef meat QMRAs was performed to give an overview of existing models. The systematic literature search was performed following the PRISMA guidelines. Articles were analysed to highlight their respective scopes, the steps in the farm-to-fork chain considered as well as implemented inputs and obtained outputs.

**Results:** QMRA models were historically developed according to specific public health concerns related to meat consumption in different countries such as Canada, Ireland or France. Thus, from 1361 articles, 136 were selected for analysis. QMRAs have been developed for a number of pathogenic bacteria species while only some considered the whole farm-to-fork chain. Articles are mainly focused either on steps at the slaughterhouse, specific beef processing steps or for retail and storage conditions. Predictive microbiology models were also considered as they greatly help to estimate the impact of growth and inactivation stages on the level of pathogens. Future challenges identified were to consider the whole farm-to-fork continuum, centralise data collection process and harmonise models developed for straightforward use and re-use in other food safety contexts.

**Conclusion and Relevance:** QMRA stands as the emerging standard for beef meat safety and – more broadly – food safety. However, models developed so far could be harmonised to consider the entire meat processing chain to facilitate their reuse to answer other specific beef safety issues. This analysis can be considered as a robust basis to integrate collected models in a harmonized tool for the whole beef meat chain and will help the scientific community and food safety authorities to identify specific monitoring and research needs.

Keywords: QMRA; beef; meat; farm-to-fork; predictive microbiology

## **87: MASSIVE OPEN ONLINE COURSE TO GLOBALLY ADDRESS LEARNING NEEDS ON THE USE OF METAGENOMICS IN ANTIMICROBIAL RESISTANCE SURVEILLANCE**

Ana Sofia Ribeiro Duarte<sup>1</sup>, Katharina D. C. Stärk<sup>2</sup>, EFFORT consortium<sup>1,3,4</sup>, EFFORT consortium<sup>5,6</sup>, Tine Hald<sup>1</sup>

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**Introduction:** One Health antimicrobial resistance (AMR) surveillance is challenged by the need to coordinate between distinct surveillance programmes from public health, veterinary and food systems. It is therefore important to develop integrated surveillance, with harmonised methods for detection of AMR determinants in humans, animals, food and the environment. The development of integrated surveillance depends on the definition of AMR itself and the choice of a quantitative measure that can be used for comparisons within and between different target populations.

**Methodology:** Metagenomics offers the possibility to monitor AMR using a universal genetics measure across surveillance targets (such as antimicrobial resistance genes (ARGs) of special interest), it is culture-independent, and it yields data in a standardised format that can be stored and shared electronically.

**Results:** The dissemination of knowledge on the potential of metagenomics for AMR surveillance is, like AMR itself, a global challenge that needs to be tackled internationally. Online open education is an effective way to disseminate knowledge at a global level. We, as part of an EU consortium working to improve surveillance and control of AMR, developed a massive open online course (MOOC) with the title '*Metagenomics applied to surveillance of pathogens and antimicrobial resistance*'.

**Conclusion and Relevance:** The course is a combination of conceptual lectures and hands-on exercises of bioinformatics and statistical analysis of metagenomics data obtained from livestock animals in nine European countries, using publicly available analytical tools. It is targeted at anyone with an interest in surveillance and control of AMR and/or an interest in learning metagenomics. The MOOC runs in 4-week sessions, which start automatically. At the time of writing (end of March 2019), it has run in 10 consecutive four-week sessions, with a total of 2006 learners enrolled (191 per session on average), 64% of which are active learners (i.e. those who have started a course item). '*Metagenomics applied to surveillance of pathogens and antimicrobial resistance*' is freely available online since June 2018 at <https://www.coursera.org/learn/metagenomics> and is rated 4.7/5 on Coursera.

**Keywords:** Metagenomics; antimicrobial resistance; surveillance; open education; e-learning

## **112: QUANTITATIVE RISK ASSESSMENT OF FUMONISINS IN CORN PRODUCED IN DIFFERENT REGIONS OF BRAZIL: INFLUENCE OF CLIMATIC CONDITIONS**

Leticia dos Santos Lopes<sup>1</sup>, Veronica Ortiz Alvarenga<sup>2,3</sup>, Fernanda Bovo Campagnollo<sup>3</sup>, Syllas Borburema Silva Oliveira<sup>3</sup>, Anderson de Souza Sant'Ana<sup>3</sup>

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**Introduction:** Fumonisins are toxins produced by fungi of the genus *Fusarium*, which are the main invaders of corn grains in the field. Fumonisins levels in corn are strongly influenced by climatic conditions. Brazil is one of the main corn producers in the world and therefore, determining the risk for fumonisins contamination is relevant to safeguard public and animal health. This study aims to determine the probability of contamination by fumonisins in corn produced in the main regions of Brazil as affected by climatic conditions in the field.

**Methodology:** The probability of occurrence of fumonisins in corn influenced by the climate conditions in the field (input - air temperature, relative humidity) was determined for the Brazilian states (RS, SC, PR, MT, MS) with the greatest corn production. The @Risk software was used to run the quantitative risk assessment model, using the Monte Carlo sampling method with 50,000 iterations.

**Results:** A strong correlation between fumonisins contamination levels and the geographic region and climatic characteristics was found, with temperature having the main influence. Regions with temperate climate, with lower average temperature and higher indices of relative humidity and precipitation, presented greater risks of occurrence (92-95%) and concentration levels of fumonisins in corn (30,000-31,000 µg/kg), in relation to tropical climate zones (58-70%; 6,800-10,700 µg/kg).

**Conclusion and Relevance:** Climatic conditions cause a strong influence on the incidence and concentration levels of fumonisins in corn produced in the main regions of Brazil. Temperature is the variable most strongly correlated with the response variable (r: -0,97; -0,61), indicating that low temperatures (19°C), associated with the high relative humidity of the air (78%), increase the probability of contamination, resulting in higher levels of fumonisins production.

**Keywords:** Climate; food security; mycotoxins; probability; risk



## **118: THE IMPACT OF A MICROBIAL REDUCTION TREATMENT ON THE RISK OF HUMAN SALMONELLOSIS FROM THE CONSUMPTION OF ALMONDS, PECANS, PISTACHIOS AND WALNUTS IN THE UNITED STATES: A COMPARISON**

Sofia Santillana Farakos<sup>1</sup>, Regis Pouillot<sup>2</sup>, Gordon Davidson<sup>1</sup>, Rhoma Johnson<sup>1</sup>, Judith Spungen<sup>1</sup>, Insook Son<sup>1</sup>, Nathan Anderson<sup>3</sup>, Jane Van Doren<sup>1</sup>

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**Introduction:** The United States is a leading producer of tree nuts worldwide. Understanding the impact of microbial reduction treatment levels and atypical situations on *Salmonella* risk of illness can inform risk management decisions on tree nut processing.

**Methodology:** We developed quantitative risk assessments to assess the risk of human salmonellosis arising from the consumption of almonds, pecans, pistachios and walnuts, separately, after the application of a *Salmonella* reduction treatment level (1-5 log CFU). These include exposure models evaluating contamination at harvest and including various steps in tree nut processing such as pre-treatment storage, post-treatment partitioning, and post-treatment and retail storage. Steps specific to each tree nut, such as immersion in water, drying, conditioning, shelling and/or cracking were included as they apply to each treat nut. U.S. consumption data and the WHO/FAO *Salmonella* dose-response model were used to assess the risk per serving and per year, quantifying variability and uncertainty separately. We modeled the impact on risk of atypical situations in the supply chain for each tree nut pre- and post-treatment as well as evaluated risk based on prevalence levels found at retail in the United States. All models were developed in R.

**Results:** Despite differences in initial contamination, survival, processing steps, and consumption, the models for almonds, pecans (cold conditioned), and pistachios estimate a minimum 4 log reduction treatment results in a mean risk of illness below one case/year in the U.S., including uncertainty. In the case of walnuts, a minimum 3-log reduction treatment would result in less than one case of salmonellosis per year under typical conditions including uncertainty. Atypical situations that may occur post-treatment (*e.g.*, cross contamination) could result in higher risk estimates that are not impacted by treatment level.

**Conclusion and Relevance:** While process control through microbial reduction treatment significantly reduces the health risk associated with consumption of tree nuts, atypical situations that occur pre- and post-treatment may lead to increased risk. The results can inform risk management decisions on tree nut processing.

**Keywords:** *Salmonella*; tree nuts; risk assessment; microbial reduction treatment; atypical situation

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## Poster Session 4: Meta-analysis Protocols and Applications; Predictive Modelling in Food Quality and Safety; and Predictive Mycology

Time: 16:10 - 16:40

Date: 19th September 2019

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### 41: INCIDENCE OF *CAMPYLOBACTER* IN CHICKEN ALONG PROCESSING AND RETAIL: A META-ANALYSIS APPROACH

Veronica Ortiz Alvarenga<sup>1,2</sup>, Leonardo Prado-Silva<sup>2</sup>, Ursula Gonzales-Barron<sup>3</sup>, Vasco Cadavez<sup>3</sup>, Anderson Sant'Ana<sup>2</sup>

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**Introduction:** Despite worldwide investment and efforts to reduce the level of contamination with *Campylobacter*, it remains at the top of all foodborne illnesses in humans and is still difficult to prevent.

**Methodology:** This study compiles and summarises published information concerning incidence of *Campylobacter* spp, *C. coli*, *C. jejuni* and *C. lari* in chicken along processing and retail. Data were extracted from 82 primary studies undertaken in Asia, Europe, Latin America, North America and Oceania.

**Results:** Overall pooled frequencies of detection found for *Campylobacter* ranged from 38.80% to 50.00%. The frequency of *Campylobacter* contamination by continents was evaluated. The higher incidence was observed in Europe (68.67%; 95% CI: 60.10 - 76.14 %) and lower incidence was found in North America (35.86%; 95% CI: 28.06 – 44.82%). In Asia, Latin America and Oceania *Campylobacter* frequencies found were of 37.40%, 54.15%, 53.03%, respectively. Also, the frequency of *Campylobacter* was inferred by stage of processing: pre-processing (defeathering, chilling), processing steps (evisceration, cutting), final products (packing, supermarket and butchering). The highest frequency was found in processing steps (61.7 %; 95% CI: 38.37 – 80.60%) followed by final product (51.1 %; 95% CI: 41.43 – 60.70%) whereas the lowest frequency was observed in the pre-processing stage (42.2 %; 95% CI: 27.34 – 69.32). The results for packing status (i.e., packed or unpacked) did not show differences on *Campylobacter* incidence which was ~48% for both. This meta-analysis also revealed that, in some European countries, the incidence of *Campylobacter* has remained high in the past 40 years.

**Conclusion and Relevance:** As expected, the middle processing – that encompasses chilling, defeathering and evisceration stages – can be considered a hotspot for *Campylobacter* contamination and spread. Thus, in order to ensure food safety, these steps

could be considered the main target for implementing hygienic procedures aiming to reduce carcass contamination.

Keywords: Foodborne; food processing; chicken carcass; evisceration; meta-analysis

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#### **114: META-ANALYSIS AND META-REGRESSION INDICATE DYNAMIC PREVALENCE AND MODERATORS OF FOODBORNE PATHOGENS IN INDIGENOUS FERMENTED MILK IN AFRICA**

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<sup>1</sup>University of Zurich, Pretoria, Switzerland. <sup>2</sup>Technical University of Denmark, Copenhagen, Denmark. <sup>3</sup>University of Zurich, Zurich, Switzerland. <sup>4</sup>University of Pretoria, Pretoria, South Africa

**Introduction:** Production of indigenous fermented milk (IFM) is mainly artisanal thus raising food safety concerns. Despite of this, the available food safety information is both limited and highly fragmented. Therefore, a meta-analysis and meta-regression were conducted to collate published data and estimate the prevalence of foodborne pathogens in African IFM.

**Methodology:** Initial search was made in Pubmed, CAB Abstracts, Web of Science and Scopus databases followed by intensive search from Google scholar, African Journals Online, and Google databases for relevant published and grey literature between years 2000 and 2017. A random effect meta-analysis model was used to determine the pooled prevalence estimates. The potential variables accounting for study heterogeneity were identified using meta-regression. Eighteen studies from 15 countries were analysed.

**Results:** The prevalence estimate for pathogenic *E. coli* was 16%, for *L. monocytogenes* 6%, for *S. aureus* 37% and for *Salmonella* spp. 3%. The pooled prevalence estimate was 12%. Heterogeneity among published articles was attributed to sampling point and microbial group, but could be moderated by year of publication, country cluster and method of microbial confirmation. The pooled prevalence was highest at milk collection points and changed over time where the odds of contamination were nine times higher in 2017 than in 2004.

**Conclusion and Relevance:** By analysing the limited number of published studies, there is an indication that IFM may be a source of major foodborne pathogens. This risk is not only high in some stages along the value chain, but recent data indicate a higher risk than past data.

Keywords: Food safety; fermented milk; meta-regression; food pathogens

## **120: A META-ANALYSIS ON HIGH-PRESSURE PROCESSING FOR *SALMONELLA* INACTIVATION IN ANIMAL AND VEGETAL PRODUCTS**

Leonardo Prado-Silva<sup>1</sup>, Verônica Alvarenga<sup>2</sup>, Bruna Castro<sup>1</sup>, Caroline Heckler<sup>1</sup>, Ursula Gonzales-Barron<sup>3</sup>, Vasco Cadavez<sup>3</sup>, Anderson Sant'Ana<sup>1</sup>

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**Introduction:** Behind the importance of *Salmonella* contamination in food safety, the consumption of fresh and healthy foods, such as meat products and fresh-cut vegetables, has become increasingly popular. To maintain the freshness without affecting the safety, the development of mild processing techniques attracts the attention of scientists and industries. Thus, high-pressure processing (HPP) has a considerable data set of pathogen inactivation kinetics available in the literature. The aim of the present study was to meta-analyse the log reductions of *Salmonella* obtained by various studies of HPP for different types of food.

**Methodology:** The study characteristics (serotype, food, intensity, time, cycles, temperature and log reduction) were extracted from primary studies published between 2004 and 2015. A total of 45 data sets of *Salmonella* serotypes inactivation from 10 different studies were collected. The type of food collected included both of animal and vegetal origin.

**Results:** The most effective HPP treatment (550 MPa) reached 8.5 and 7.09 log reduction for ground chicken at 4°C and tomato at 20°C, respectively. On the other hand, for the same products and temperature conditions, the least effective inactivation was found for ground chicken (-0.50 log) and tomato (-0.46 log) applying 250 MPa and 350 MPa, respectively. Among all data collected, an average value of 4.12 log reduction was reached for animal-origin products (N=32) and 3.91 log reduction for vegetal-origin products (N=13). The meta-analysis produced a between-study heterogeneity  $\tau^2$  of 0.1780 with a low value of intra-class correlation (30.64%).

**Conclusion and Relevance:** This study for the first time integrates all data available on the impact of HPP as a *Salmonella* inactivation method among a diversity of food products from animal and vegetal origin.

**Keywords:** *Salmonella*; emerging technology; inactivation; foodborne disease

## 121: A SQUARE-ROOT META-REGRESSION MODEL DESCRIBING THE GROWTH OF *LISTERIA MONOCYTOGENES* IN MEAT

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<sup>1</sup>CIMO Mountain Research Centre, School of Agriculture, Polytechnic Institute of Bragança, Bragança, Portugal. <sup>2</sup>Nestlé Research Centre, PO BOX44, CH-1000, Lausanne, Switzerland

**Introduction:** Many researchers have assessed the kinetics of *Listeria monocytogenes* (LM) in several meat matrices, and in this study, the objective was to integrate all published results on LM growth in meat by constructing a meta-regression based on the square-root model.

**Methodology:** Suitable primary studies were identified through systematic literature search. From the 45 studies considered appropriate for inclusion, information on the study ID, specific growth rate (GR), temperature (T), pH,  $a_w$  and type of meat was obtained. The meta-analytical square-root model for suboptimal temperatures [ $\sqrt{\text{GR}} = b(T - T_{\min})$ ;  $T_{\min}$ : theoretical minimum temperature for growth] was adjusted to the data set as a mixed-effects regression with type of meat affecting  $b$  and  $T_{\min}$ , and placing random effects due to primary study in  $b$  and  $T_{\min}$ .

**Results:** Type of meat consisted of: beef (N=107; beef fillet, ground beef), pork (N=36; loin chop, ground pork), poultry (N=78; breast, thigh, turkey, chicken broth and chicken nuggets), processed meat (N=16; cooked ham and pate), sausage (N=61; dried, salami, bologna), cooked beef (N=152), cooked poultry (N=12) and non-specific meats (N=84). The overall constant  $b$  was estimated at 0.0226 1/(°C√h) (SE: 0.0014 1/(°C√h)) and the overall  $T_{\min}$  at -2.966°C (SE=0.0162 °C). Type of meat turned out to be a moderator of  $b$  ( $p < .0001$ ) and  $T_{\min}$  ( $p < .0001$ ), explaining ~40% of the between-study variability  $\tau^2$ , which accounted for ~71% of the total variability. The results suggested that, for the same increase in temperature, LM in pork ( $b=0.0177$ ) would grow slower than in cooked beef ( $b=0.0256$ ) or processed meat ( $b=0.0244$ ). The lowest  $T_{\min}$  values were found for cooked beef (-4.093°C) and processed meat (-3.933°C), while pooled  $T_{\min}$  for beef (-2.633°C), poultry (-2.483°C), cooked poultry (-2.834°C), pork (-1.560°C) and non-specific meats (-2.888°C) did not differ significantly from one another. A separate nested random-effects model proved the underlying dependence of  $b$  and  $T_{\min}$ , estimating a correlation of 0.756 between their meat-specific deviations.

**Conclusion and Relevance:** The parameters meta-analysed can be used as reference values in quantitative risk assessment. Moreover, this type of model can also help in the differentiation of food classes according to their susceptibility to LM growth.

**Keywords:** Meta-analysis; secondary model; *Listeria monocytogenes*; suboptimal; food

### 135: A META-ANALYSIS ON THE TENDERISING EFFECT OF AGEING BEEF MUSCLE

Vasco Cadavez<sup>1</sup>, Calvin Van Velthoven<sup>2</sup>, Maikel Maloncy<sup>2</sup>, Ursula Gonzales-Barron<sup>1</sup>

<sup>1</sup>CIMO Mountain Research Centre, Polytechnic Institute of Bragança, Bragança, Portugal. <sup>2</sup>The Hague University of Applied Sciences, The Hague, Netherlands

**Introduction:** While tenderness is the most important quality trait of meat, it is a property affected by multiple factors; namely, animal breed, sex and age, pre-slaughter stress, muscle type, meat's ultimate pH, carcass tenderisation, cooling profile and ageing. The objective of this work was to understand, through meta-analysis, how intrinsic and extrinsic factors modulated the effect of ageing time on beef meat tenderness, as measured by the Warner-Bratzler (WB) shear-force (SF).

**Methodology:** After methodological quality assessment of studies retrieved from Scopus and PubMed, forty studies were kept for meta-analysis, from which the following information was extracted: animal gender, age class, breed, muscle type, carcass weight, meat's ultimate pH, pre-treatment of meat sample before SF measurement (chilled vs. frozen), ageing time, SF mean and standard error, WB crosshead speed and WB shear area (1.13-1.77 cm<sup>2</sup> for cylinder- and 1 cm<sup>2</sup> for parallelepiped-shaped subsamples). A meta-analysis model was adjusted to a data set consisting of 462 SF values as a mixed-effects linear regression testing for the effects of intrinsic/extrinsic factors and ageing time on SF.

**Results:** Ageing strongly favours tenderisation of meat ( $p < .0001$ ); however, ageing time has an accelerating tenderisation effect during ~2-3 weeks, after which the increase in tenderness slows down. Beef tenderness is also affected by cattle breed, having *Bos indicus* a tendency to produce tougher meat ( $p < .0001$ ) than *Bos taurus*. The type of muscle exerts an effect on tenderness ( $p < .0001$ ): *Psoas major* and *Longissimus lumborum* produce the most tender beef meat, and are significantly different from *L. thoracis*, which has an intermediate tenderness. *Semimembranosus* and *Semitendinosus* muscles produce the toughest meat. Freezing of meat, and posterior defrosting for analysis, has also a negative impact on tenderness ( $p = 0.0060$ ). Finally, SF measurements are affected by meat subsample shape ( $p = 0.0098$ ), crosshead speed ( $p = 0.0006$ ) and their interaction ( $p = 0.0002$ ).

**Conclusion and Relevance:** On average, the minimum ageing time for meat tenderisation at very low refrigeration temperatures is 2 weeks. Furthermore, this meta-analysis demonstrated the importance of standardising the measurement of WB SF, since higher crosshead speeds and the lowest shear areas of 1x1 cm<sup>2</sup> from parallelepiped-shaped meat subsamples produce lower SF values.

**Keywords:** Warner-Bratzler; shear-force; tenderness; maturation; meat

## 18: SCREENING OF DIFFERENT CONDITIONS LEADING TO THE EXOPOLYSACCHARIDES PRODUCTION BY *ENTEROCOCCUS FAECIUM* F58

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**Introduction:** Lactic acid bacteria (LAB) were widely used as starter cultures to improve the safety and the quality of food products. *Enterococci* are an important part of the natural LAB consortium. They have been generally recognized as safe (GRAS). The application of enterococcal molecules secretion such as exopolysaccharide (EPS), lactic acid, and bacteriocins is promoted in diverse sectors. EPS are ones of the most biomacromolecules used in food industry. They are economically significant, because they can impart functional effect to food products and confer beneficial health effect on human when consumed. The main objective of this work was to screen how the variables: tween 80, MnSO<sub>4</sub>, H<sub>2</sub>O, K<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub>.7H<sub>2</sub>O, sodium acetate, yeast extract, maltose, pH, temperature, time, and inoculum, affected the production of EPS produced by *E. faecium* F58, which was isolated from goat's fresh cheese (jben) and well known to produce L50A and L50B enterocins.

**Methodology:** A Plackett-Burman design was used to screen, among eleven variables, which of them exhibited high impact on EPS yield of *E. faecium* F58 strain. An analysis at three levels: high (+1), medium (0), and low concentration (-1) were performed for each variable resulting in 15 trials.

**Results:** The results showed that the significant variables that affected EPS yields of *E. faecium* were MnSO<sub>4</sub>, H<sub>2</sub>O, yeast extract, and time. The data analysis showed that P-values were less than 0.050, which indicated that the model terms were all significant.

**Conclusion and Relevance:** In this study, *E. faecium* showed the ability to produce EPS yields from 0.15 to 65.34 g/L under different conditions of MnSO<sub>4</sub>, H<sub>2</sub>O, yeast extract and time. The results obtained in this study suggested that *E. faecium* F58 could become a new source for the production of EPS used in food industry.

**Keywords:** Lactic acid bacteria; Plackett-Burman design; *Enterococci*

### 33: INTEGRATION OF *LISTERIA* spp. GROWTH RATE VARIABILITY AND TEMPERATURE DISTRIBUTION IN DOMESTIC SCENARIO TO PREDICT CHANGES OF SHELF LIFE

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**Introduction:** *Listeria monocytogenes* is a food borne pathogen that can grow at usual refrigerating temperatures exceeding the EC regulation limit of 100 CFU/g (EC 2073/2005) during the shelf life. Specific objectives of this study were: (a) to study the growth variability of *Listeria* spp. isolated from food (b) to monitor domestic refrigerator temperatures and (c) to use the mathematical models to predict the *Listeria* spp. growth in dynamic domestic scenario.

**Methodology:** A total of 22 wild strains of *Listeria* spp. were cultivated in BHI broth at 25°C. Data were fitted to a sigmoidal Baranyi model by using DMFit obtaining the growth rates. Using the maximum (rate<sub>max</sub>) and minimum (rate<sub>min</sub>) growth rates as reference rates, the secondary model of Ratkowsky was used to model the growth as a function of the temperature. A national survey plan on 800 Italian families has been performed to monitor the temperature of domestic refrigerator on top, middle and bottom shelves for 5 days. The Runge–Kutta method was used to predict the *Listeria* spp. growth during a representative domestic scenario.

**Results:** Wide strains variability was found in relation to the kinetics properties: at 25°C the growth rates ranged from 0.27 h<sup>-1</sup> (rate<sub>min</sub>) to 0.37 h<sup>-1</sup> (rate<sub>max</sub>). The representative domestic refrigerator temperature used in this study ranged from -6°C to 8°C (top), +4.5 °C to 9°C (middle) or from -1.5°C to 9.5°C. The integration of these parameters allow to predict the pathogen growth: starting from 1 log CFU/ml, the time to reach the limit of 2 log CFU/ml was 100 h (top), 65 h (bottom) or 50 h (middle) considering the rate<sub>max</sub> as a reference rate in Ratkowsky model, while the limit was not reached considering the rate<sub>min</sub> of *Listeria* spp.

**Conclusion and Relevance:** The wide variability of *Listeria* spp. growth underlines the importance to consider the worst scenario to reduce the risk for the consumers. In addition, it is need to improve consumer education regarding the importance of being aware of their fridge temperature, emphasizing the fact that different zones within each refrigerator have different temperatures.

**Keywords:** *Listeria monocytogenes*; storage temperature; domestic refrigerator; food safety



## 45: NON-INVASIVE MULTISPECTRAL IMAGE ANALYSIS FOR THE ASSESSMENT OF SPOILAGE IN POULTRY PRODUCTS

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**Introduction:** Multispectral image analysis is considered as a potential tool for rapid and non-invasive evaluation of the quality of meat products. This method has been applied on a variety of meat products resulting in the development of spoilage predictive models. The aim of this study was to correlate microbiological and multispectral data acquired from at-line measurements in a meat processing factory for two poultry products. Partial Least Squares-Regression (PLS-R) analysis was further undertaken for the evaluation of the microbiological quality of these products.

**Methodology:** Fillets of chicken thigh (n=97) and chicken burger (n=131) were analyzed microbiologically for the determination of total viable counts (TVC) and *Pseudomonas* spp. as well as with multispectral imaging (wavelengths: 405-970 nm). In addition, samples were stored at isothermal conditions (4 °C for 216 h) and subjected to microbiological and multispectral image analyses. PLS-R analysis was applied for the estimation of TVC, *Pseudomonas* spp. and time from slaughter. PLS-R was performed using the Unscrambler v. 9.07 software and the optimum number of latent variables were defined using leave-one-out cross validation.

**Results:** For the estimation of TVC, PLS-R models for chicken thigh were successfully developed with RMSE and R<sup>2</sup> values of 0.288 and 0.965, respectively, while for chicken burger the respective values were 0.406 and 0.958. In addition, *Pseudomonas* spp. counts were satisfactorily predicted, since for chicken thigh RMSE and R<sup>2</sup> values were 0.314 and 0.967, respectively, whereas for chicken burger they were 0.327 and 0.924. Finally, models for time from slaughter showed RMSE and R<sup>2</sup> values of 0.097 and 0.904, respectively, for chicken thigh, while for chicken burger the calculated values were 0.051 and 0.969.

**Conclusion and Relevance:** The application of these findings in the production and/or distribution line could assist for the proactive assessment of poultry products quality.

**Acknowledgement:** This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-04344)

**Keywords:** Poultry products; multispectral imaging; *Pseudomonas* spp.; spoilage assessment; rapid methods

## 46: RAPID QUALITY ASSESSMENT OF POULTRY PRODUCTS USING AT-LINE MULTISPECTRAL IMAGING (MSI)

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**Introduction:** In the last decade non-destructive methods have been implemented in an effort to eliminate food waste due to spoilage while predictive models assessing meat quality have been developed using data from multispectral images. The aim of this study was to correlate microbiological and multispectral data collected from at-line measurements for two poultry products and perform Partial Least Squares (PLS-R) analysis for the estimation of their microbiological quality.

**Methodology:** Samples of chicken breast fillets (n=107) and marinated chicken souvlaki (n=134) were selected from the production process and analyzed microbiologically for the enumeration of total viable counts (TVC) and *Pseudomonas* spp. In parallel, spectral images (wavelengths: 405-970 nm) were obtained using a MSI instrument. Moreover, samples were stored at 4 °C for 216 h and subjected to microbiological and multispectral analyses. Partial Least Squares-Regression (PLS-R) analysis was conducted from both datasets for the assessment of TVC, *Pseudomonas* spp. and time from slaughter. PLS-R was performed using the Unscrambler v. 9.07 software and the optimum number of latent variables were defined using leave-one-out cross validation.

**Results:** PLS-R models for TVC presented good performance. Specifically, for chicken breast RMSE and R<sup>2</sup> values were 0.342 and 0.927, respectively, while for marinated chicken souvlaki RMSE and R<sup>2</sup> were 0.505 and 0.872. For *Pseudomonas* spp. counts, the respective values of RMSE and R<sup>2</sup> for chicken breast were 0.342 and 0.928, respectively, whereas for marinated chicken souvlaki they were 0.661 and 0.841. Furthermore, for the estimation of time from slaughter, the values of RMSE and R<sup>2</sup> were 0.077 and 0.939 as well as 0.102 and 0.907 for chicken breast and marinated chicken souvlaki, respectively.

**Conclusion and Relevance:** PLS-R models could be successfully implemented in tandem with Multispectral analysis for the assessment of microbiological quality in poultry products.

**Acknowledgement:** This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-04344)

**Keywords:** Multispectral imaging; poultry products; spoilage; at-line measurements; rapid techniques

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## 47: OPTIMISATION OF BENZALKONIUM CHLORIDE TREATMENT IN THE DISINFECTION OF *LISTERIA MONOCYTOGENES* IN THE FOOD INDUSTRY

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**Introduction:** *Listeria monocytogenes* is a significant concern in the food industry. It is a foodborne gram-positive pathogenic bacterium responsible for human listeriosis. Although food equipment and contact surfaces are cleaned and disinfected routinely, *L. monocytogenes* can grow and persist in harbourage sites. Besides, the use of biocides such as benzalkonium chloride (BAC), may induce resistance or cross-resistance to other antimicrobials such as antibiotics. Therefore it is of the highest interest, to define disinfection protocols that may reduce, if not eliminate, its presence in the industrial plants but also avoid the emergence of resistance. In this work, we present a model-based design of the disinfection protocols.

**Methodology:** We formulate a novel mechanistic model which accounts for inoculum and BAC decay kinetics, by taking into consideration the effect of inoculum size and both adsorption and penetration of BAC within the cells. The model consists of 4 non-linear ordinary differential equations. We estimated model parameters through multi-experiment data fitting using AMIGO2 toolbox ([sites.google.com/site/amigo2toolbox/](https://sites.google.com/site/amigo2toolbox/)). Data for modelling were obtained from times series data collected under different inocula (6-10 log), and BAC doses (40- 60 ppm). We used the fitted model to compute the dosage profile to minimise sublethal treatments while reducing the use of BAC.

**Results:** The proposed model explains all data sets while outperforming previous models found in the literature ( $R^2 > 0.97$ ). Its mechanistic nature brought novel insights into the way BAC acts at the level of the cellular membrane. The disinfection operates in two steps, a first step, in which BAC disrupts the membrane, and a second step, in which BAC penetrates the cell. The model quantifies the dynamics of both mechanisms.

**Conclusion and Relevance:** This work proposed and validated a mechanistic dynamic model which explains the disinfection of *L. monocytogenes* by BAC. The model explicitly accounts for membrane processes and can be directly applied to other gram-positive and gram-negative pathogenic bacteria; therefore, opening new venues for the optimal design of disinfection protocols in the food industry.

**Keywords:** Food industry; inactivation kinetics; antimicrobial resistance; chemical disinfection; inoculum effect.

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## 62: COMBINATON OF FINITE ELEMENTS METHOD (FEM) AND KINETIC MODELS TO SIMULATE MICROBIAL INACTIVATION DURING COOKING OF A SOLID FOOD PRODUCT

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**Introduction:** Predictive models to ensure safety during cooking has gained popularity recently. However, due to the thermal inertia of solid food products, the temperature is heterogeneous during cooking. Most models overcome this issue using the assumption that temperature in the whole product is the one of its coldest point. This results in overprocessing, which may have a negative impact on sensorial quality. The aim of this research was to develop a kinetic model for microbial inactivation in chicken that takes into account the temperature distribution in meat during cooking.

**Methodology:** Chicken samples were vacuum packed, inoculated with *Listeria monocytogenes* and heat treated in a water bath. Then *L. monocytogenes* was enumerated by plating in selective medium. Two mathematical models were developed: -A kinetic one for microbial inactivation, based on the Geeraerd microbial inactivation model. An experimental protocol was developed that minimized the effect of the thermal heterogeneities, allowing a characterisation of the thermal inactivation kinetics in this medium. -A Finite Elements Method (FEM) model considering the fluid flow and the thermal exchange (fluid-fluid, fluid-food, food-food). Both models were validated independently. Then, they were combined to predict microbial inactivation during cooking taking into account the temperature distribution. The combined model was also validated.

**Results:** The experiments enabled to predict the thermal inactivation of *L. monocytogenes* in the food analysed. Also, the FEM model could accurately predict the (heterogeneous and dynamic) temperature in meat during cooking. The combination of both models enabled to predict the level of microbial inactivation attained during cooking of portions with spherical shape.

**Conclusion and Relevance:** The protocol (experimental and computational) proposed in this study was successful at describing the relevant factors; i.e. the temperature heterogeneities and the microbial inactivation. The models developed can, thus, be applied to optimise cooking conditions for minimally processed food products. The models developed can be applied to ensure the security of thermal processes in the meat product analysed. This shall increase consumer safety and may result in process optimisation. Also, this study can serve as guideline to future, similar studies, where the heterogeneities of solid food matrixes are taken into account for process design.

**Keywords:** Microbial inactivation, thermal treatment, temperature distribution, *Listeria monocytogenes*, food safety

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## 94: PROFILE LIKELIHOOD-BASED PREDICTION CONFIDENCE INTERVALS USING QUASE-MONTE CARLO

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**Introduction:** Predict the shelf life of food products, mathematical modelling of microbial growth with a suitable estimation of parameters is essential. However, the uncertainty of the experimental data has a significant impact on the model predictions. Thus, determining the identifiability of the parameters and dealing with uncertainties will be principally the most important concerns to cope with.

**Methodology:** In order to determine the prediction confidence intervals considering parameter uncertainties, we propose a quasi-Monte-Carlo approach. We apply the maximum likelihood estimation (MLE) method to the modified-Gompertz model as the primary model, combined with secondary models for the temperature dependence of the model parameters. In contrast to the standard way we estimate all model parameters in one multi-experiment fit, which yields better results for the overall model prediction. After estimating parameters and their confidence intervals using profile likelihood, we calculate the predictions confidence intervals by a quasi- Monte-Carlo method.

**Results:** To evaluate the performance of the proposed method, we use the raw data for growth of total viable count in pork loin under MA-packed conditions for five different temperatures 2, 4, 7, 10 and 15 °C. The results show that the modified-Gompertz model parameters are identifiable in the presence of experimental data. We show that the proposed method yields reliable calculations of confidence intervals in comparison to the widely accepted standard method of interval construction. We also show that the standard method does not provide correct confidence intervals, and this may have an impact on analyses that incorporate model prediction uncertainty.

**Conclusion and Relevance:** In this study, a quasi-Monte-Carlo approach is applied to measure the model prediction uncertainty. The results show that the proposed method yields reliable calculation of the model prediction uncertainty and addresses a particular shortcoming of the standard method for constructing confidence intervals. The proposed method provides an efficient and easy to implement algorithm for uncertainty quantification in predictive food modelling.

**Keywords:** Identifiability; uncertainty quantification; quasi-Monte Carlo approach; profile likelihood

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## 98: EFFECT OF MICROBIAL SANITISERS AND SILVER NANOPARTICLES ON ERADICATION OF *LISTERIA MONOCYTOGENES* AND *SALMONELLA* BIOFILMS

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**Introduction:** Biofilm formation represents a serious concern in many milk factories causing microbial contamination of dairy products. This study aims to investigate the effect of different disinfectants (silver nitrate (0.5, 1 & 1.5%), ethanol (2.5, 5, 7.5 & 10%), sodium hypochlorite (1, 5 & 10%), hydrogen peroxide (2, 4 & 6%), peracetic acid (0.5, 5, 10 & 15%)) and silver nanoparticles (5, 10, 25, 50, 75 & 100 µg/ml) at different contact times (1, 5, 15, 30 & 60 min) on a developed 24h- biofilm of *Listeria monocytogenes* and *Salmonella* spp strains.

**Methodology:** Three strains of *L. monocytogenes* and two strains of *Salmonella* were examined for biofilm formation in a 10x10 microtiter plate in Bioscreen C at 37°C for 24h. Biofilm quantification was performed using BHI and TSB for *Listeria* and *Salmonella*, respectively. The obtained biofilm was fixed by methanol and stained with crystal violet 2%, then glacial acetic acid was added to re-solubilise the staining solution. Finally, the efficiency of the disinfectants and nanoparticles on eradication of *L. monocytogenes* and *Salmonella* biofilms was evaluated at 1, 5, 15, 30- and 60-min using crystal violet assay.

**Results:** The amount of biofilm produced by *Salmonella* spp. (OD; 0.440) was greater than that produced by *L. monocytogenes* (OD; 0.202). Sodium hypochlorite (5%) showed the highest inhibitory effect against biofilm formation since it could remove 69.5% of *Salmonella* biofilm when applied for 60 min., while, peracetic acid (5%) and hydrogen peroxide (6%) decreased in 30.4% and 25.6% the biofilm formation, respectively. Regarding the application of silver nanoparticles, the highest inactivation (38.4%) was observed at 25 µg/ml for 30 min. Additionally, silver nitrate at all concentrations did not show any significant effect against biofilms.

**Conclusion and Relevance:** The ability of *Salmonella* to produce biofilm on surfaces was higher than for *L. monocytogenes*. Furthermore, sodium hypochlorite was more effective against *Salmonella* biofilm and can be used as a biofilm removal sanitiser in milk industries to avoid contamination of dairy products. The current study could provide food producers with useful information about the most effective way to eliminate the biofilm produced by *L. monocytogenes* and *Salmonella* spp.

**Keywords:** Biofilm; *L. monocytogenes*; *Salmonella*; sodium hypochlorite; silver nanoparticles

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**125: EFFECT OF CAMU-CAMU (*MYRCIARIA DUBIA*) POWDER ON LIPID OXIDATION AND KINETICS OF *SALMONELLA* TYPHIMURIUM AND SPOILAGE BACTERIA IN VACUUM-PACKED GROUND BEEF**

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**Introduction:** Camu-camu is an Amazonian berry fruit with documented bioactive properties. This study aimed to assess the effects of camu-camu powder (CCP) on lipid oxidation and kinetics of *Salmonella* Typhimurium and spoilage bacteria in vacuum-packed ground beef.

**Methodology:** Batches of 200-g ground beef were mixed with 0%, 2.0%, 3.5% and 5.0% CCP, vacuum-packed in 10-g portions, and stored at 4°C. Psychrotrophic (PSY) and lactic acid bacteria (LAB) were counted on days 0, 3, 6, 9, 13 and 16; while TBARs and pH were quantified on days 1, 7 and 15. Another experiment was conducted with the same camu-camu doses, yet inoculating ~5 log CFU/g *S. Typhimurium* (ST). Experimental microbial kinetic curves were modelled by the Huang growth and the Weibull decay models. Physicochemical properties were analysed by ANOVA.

**Results:** Higher doses of CCP were found to progressively slow down the growth of LAB from 0.596 log CFU/d (2.0% CCP) until 0.349 log CFU/d (5.0% CCP). Similarly, the growth rates of PSY in ground beef with added CCP were lower (0.788 - 0.912 log CFU/d) than in the control (1.210 log CFU/d). The lag phase of PSY in meat increased with higher doses of CCP, from 7.438 d (2.0% CCP) until 8.156 d (5.0% CCP). The addition of CCP at any dose produced a steeper inactivation of ST during the first days of cold storage, as represented by the Weibull's concavity shape  $\alpha = 0.371-0.514$ , in contrast to 1.240 for the control; however, at the end of the experiment, ST counts in meat with CCP were not different from the control (4.610 - 4.796 log CFU/g). Although the addition of CCP increased the initial meat pH, on day 15 there were no differences between treated samples and control. CCP caused a quick reduction of TBARs in meat from 3.144 until 0.007-0.117 mg MDA/kg, and even at the lowest CCP dose of 2.0% and the longest storage, the TBARs only reached 0.650 mg MDA/kg.

**Conclusion and Relevance:** Although CCP affects bacterial kinetics, it does not protect ground beef against spoilage bacteria and *Salmonella* to the same degree it does against lipid peroxidation.

**Keywords:** Psychrotrophic; lactic acid bacteria; TBARs; mince beef

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## 151: SURVIVAL OF *STAPHYLOCOCCUS AUREUS* ON VACUUM-PACKED BEEF STEAKS COATED WITH ANNATTO (*BIXA ORELLANA* L.) SEEDS OIL-EXTRACT

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**Introduction:** In Latin America, seeds of annatto (*Bixa orellana* L.) have been traditionally used in cookery as a condiment and colouring agent, although medicinal properties have been also attributed to its leaves and seeds. Although annatto seed extracts, rich in bixin and norbixin, are better known in the food industry as colourant additives, these extracts have been also demonstrated to have *in-vitro* antimicrobial activity against *Staphylococcus aureus* (minimum inhibitory concentration ~62.5 µg/ml). The objective of this study was to investigate the survival of *S. aureus* on vacuum-packed beef steaks coated with annatto seeds oil-extract throughout cold storage.

**Methodology:** Ten g of powdered annatto seeds was extracted with 100 ml oil in agitation for 8 hours at 55°C. The oil was cleared through sequential centrifugation and filtration. Annatto extracts contained <1.0% bixin. *S. aureus* in log phase was surface-inoculated on beef steaks of 40 cm<sup>2</sup> to reach ~3.50 log CFU/cm<sup>2</sup>, and allowed to dry. In the control treatment (Control), inoculated beef steaks were individually vacuum-packed. For the other treatments, beef steaks in pouches were coated with 1-ml oil (Oil) or with 1-ml annatto oil-extract (Bixin), and vacuum-packed. All samples were stored at 5°C for 12 days. Typical colonies of *S. aureus* were counted on YET-supplemented Baird-Parker agar on day 0 and every 2-3 days. A Weibull decay ( $\beta$ ,  $\sigma$ ) model with shape parameter  $\beta$  and time  $\sigma$  to achieve first decimal reduction was fitted to each survival curve.

**Results:** In terms of shape, the Control curve was concave ( $\beta=0.698$ ) while the curves from both the Oil and Bixin treatments were convex ( $\beta=1.038$  and  $1.029$ , respectively). However, the main difference among treatments was evident in the parameter  $\sigma$ , suggesting that coating with annatto oil-extract ( $\sigma=5.45$  days) significantly reduced the time to achieve the first decimal reduction by more than a half, when compared to the Control ( $\sigma=13.76$  days) and Oil ( $\sigma=13.36$  days) treatments. Thus, after 12 days of cold storage, beef steaks with annatto oil-extract presented *S. aureus* counts of  $1.34\pm0.638$  log CFU/cm<sup>2</sup>, while higher counts were recovered from the untreated steaks ( $2.86\pm0.406$  log CFU/cm<sup>2</sup>) and those coated only with oil ( $2.64\pm0.213$  log CFU/cm<sup>2</sup>).

**Conclusion and Relevance:** In 12 days, vacuum-packing and cold storage reduced *S. aureus* counts on beef steaks in  $0.721\pm0.673$  log CFU/cm<sup>2</sup>. If annatto oil tincture is added as a coating, the reduction is significantly higher at  $2.138\pm0.494$  log CFU/cm<sup>2</sup>. Hence, in addition to the flavour enhancing properties of annatto seeds, these results suggest that annatto oil-extract has also the ability to inhibit *S. aureus*, and therefore can be used as condiment coating on beef steaks.

Keywords: Antimicrobial; bixin; Weibull model; oil-based coating



### 38: GROWTH KINETICS OF THREE *SACCHAROMYCES CEREVISIAE* STRAINS USED IN FERMENTATION PROCESSES IN THE PRESENCE OF DIFFERENT OCHRATOXIN A CONCENTRATIONS

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**Introduction:** Yeasts can have a role in biodegradation of ochratoxin A (OTA) during wine-making. Despite this, it is not known whether the presence of this mycotoxin can influence on the performance of yeasts during the fermentation steps. In this sense, this study aimed to determine the influence of the ochratoxin A presence on the growth kinetics of *Saccharomyces cerevisiae* strains.

**Methodology:** Three *S. cerevisiae* strains (12M, 01PP, 41PP) ( $5 \times 10^5$  cells/mL) commonly employed for wine-making were inoculated, individually, in broth containing 6.7 % yeast nitrogen base, 20 % glucose, 0.1 % diammonium phosphate and adjusted to pH 3.6 with tartaric acid. The fermentation broth was spiked with OTA at concentrations of 10, 20 and 30  $\mu\text{g/L}$ . A control (no OTA) was performed for each strain. The fermentation broth was incubated at 25 °C and samples were collected at 0, 2, 4, 7, 10, 16, 22, 28, 38, 48 e 58 h of fermentation for growth evaluation. The Baranyi model was fitted to the growth data (log CFU/mL).

**Results:** OTA presence in the concentrations tested did not influence the growth of the *S. cerevisiae* strains studied ( $p < 0.05$ ). All strains showed an increase of 2 log cycles ( $10^7$  cells/mL) throughout the fermentation process. Despite this, strain 12M presented longer exponential and stationary phases, reaching 58 h of growth, whereas strains 01PP and 41PP enter the decline phase after 28 h of fermentation. Strain 12M showed a lower growth rate ( $\mu$ ) ( $0.08\text{-}0.12 \text{ h}^{-1}$ ) and a higher lag phase ( $\lambda$ ) ( $3.81\text{-}5.98 \text{ h}$ ) when compared to strain 01PP ( $\mu$ :  $0.19\text{-}0.23 \text{ h}^{-1}$ ;  $\lambda$ :  $1.53\text{-}2.41 \text{ h}$ ) and 41PP ( $\mu$ :  $0.15\text{-}0.18 \text{ h}^{-1}$ ;  $\lambda$ :  $1.10\text{-}2.64 \text{ h}$ ). The maximum population ( $R_g$ ) was similar among the strains in the presence of OTA, while in the control assays, strain 12M showed a higher maximum population ( $R_g$ :  $7.43\text{-}7.64 \text{ log CFU/mL}$ ) than strain 01PP ( $R_g$ :  $7.24\text{-}7.39 \text{ log CFU/mL}$ ) and 41PP ( $R_g$ :  $7.25\text{-}7.46 \text{ Log CFU/mL}$ ).

**Conclusion and Relevance:** Although some fungal metabolites may affect yeast performance in industrial fermentations, the yeast strains studied were not affected by OTA. Probably the tested yeast strains can adapt to the presence of OTA.

**Acknowledgements:** FAPESP, Grant #2016/21041-5.

**Keywords:** Baranyi model; growth; modelling; mycotoxin; wine-making

## **48: AN APPROACH TO PROVIDE SAFE RECOMMENDATIONS TO THE CONSUMERS WITH RESPECT TO MOULDY PRODUCTS**

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**Introduction:** In the farm to fork approach, the consumer can be considered as an important element of the chain. Mould contamination is easily detected by the consumer due to the presence of a noticeable mycelium. The main problem with mould spoiled foods is the possible production of mycotoxins that can diffuse from the mycelium to the product. However, the studies on this topic are scarce.

**Methodology:** The objective of this lecture is to list all the studies assessing the ability of mycotoxins to diffuse in food products. Mouldy food products such as soft cheese, meat, poultry should be discarded because they can also be contaminated by bacteria. Therefore, the approach applied to low water activity food, and fruits because they are dry and/or acid enough to prevent from bacterial growth. It is explained which isolates and which mycotoxins should be selected, how food should be inoculated to reproduce real contamination, how sampling should be performed.

**Results:** The contamination of a semi-hard cheese by *Penicillium verrucosum* is used as an example to assess migration of mycotoxins. The aspect and the size of the mouldy part of the food which are the only available information for the consumer were correlated to the concentrations of citrinine, CIT and ochratoxin A, OTA in cheese. As long as white mycelium only is visible on cheese, trimming the first mm of the cheese is acceptable, but any "blue moulded" cheese should be discarded because toxins can be detected up to 16 mm depth.

**Conclusion and Relevance:** Consumer's knowledge on the ability of some moulds to produce carcinogenic toxins in food products is weak. Accurate information should be provided to the consumer to ensure food safety, and to reduce food losses. Recommendations should also be given at the retail stage.

Keywords: Mycotoxins; food safety; predictive mycology; *Penicillium*

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### **73: MODELLING THE ANTIFUNGAL ACTIVITY OF MEXICAN OREGANO (*LIPPIA BERLANDIERI* SCHAUER) ESSENTIAL OIL INCORPORATED TO ALGINATE EDIBLE FILMS IN TOMATO STEMS**

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**Introduction:** Edible coatings provide a semi-permeable barrier to gases and water vapor, and their protective function can be enhanced with addition of natural antimicrobials. In order to observe and model the antifungal effect of Mexican oregano essential oil (EO) incorporated into alginate (1.5%) edible films with selected EO concentrations (0, 0.25, 0.75, 1, 2, or 4% v/v) to cover tomato stems inoculated with *Penicillium commune*, *Fusarium* spp., or *Cladosporium herbarum* were prepared.

**Methodology:** Tomatoes were treated in two different ways; TIC treatment consisted of previously inoculating the tomato (10 $\mu$ L of spore suspension) in the stem and subsequent coating with alginate-essential oil films. The second treatment, TCI, consisted of pre-coating tomato with alginate-essential oil films, and subsequently inoculating the tomato (10 $\mu$ L of the spore suspension) at the stem. In every case, tomatoes were stored under refrigeration (4°C), and observed daily for 21 days; mould's growth or no growth on the surface of the product was recorded, as well as the time at which mould growth was detected. In order to model the effect of the applied treatments, mathematical models based on the time-to-fail and binary logistic regression were proposed.

**Results:** For TCI treatment, *P. commune* and *C. herbarum* were inhibited with 2% of oregano EO edible films; while *Fusarium* spp. growth was detected. Regarding TIC treatment, films added with 0.75% or 1% EO were effective against every studied mould. For TCI with 0.75% of EO, time-to-fail model predicted a shelf life of 24, 11, or 17 days for tomatoes inoculated with *P. commune*, *C. herbarum*, or *Fusarium* spp., respectively; this was consistent with the probability model, which estimated a probability of growth of 0.79, 0.57, or 0.70, respectively. In the case of TIC, predicted times to failure for tomatoes with a film with 0.75% EO were 20, 17, or 14 days, with a probability of growth of 0.88, 0.79, or 0.70, respectively.

**Conclusion and Relevance:** The application of alginate films made without Mexican oregano essential oil to tomatoes did not inhibited mould growth. TCI treatment, which simulates the possible external contamination of tomatoes, meaningfully slowed down mould growth thus could be utilised to importantly reduce fruit losses.

**Keywords:** Tomatoes; logistic regression; time-to-fail-models; oregano essential oil; alginate edible films

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### 93: MODELLING ASCOSPORES INACTIVATION OF *BYSSOCHLAMYS NIVEA* AND *BYSSOCHLAMYS FULVA* IN CLARIFIED APPLE JUICE BY UV- C LIGHT

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**Introduction:** Ascospores of *Byssochlamys nivea* and *Byssochlamys fulva* are heat-resistant and can survive thermal commercial treatments normally applied to the juices, as apple juice. UV- C light is a non-thermal technology with potential application in food industries to provides an alternative to thermal pasteurisation. The aim of this study was to model the inactivation of *B. fulva* and *B. nivea* ascospores in apple juice by UV-C light.

**Methodology:** UV-C treatments were carried out in a camera with germicidal lamps (peak emission at 254 nm). For the experiments, aliquots (0.1 mL) of each ascospores suspension (*B. nivea* and *B. fulva*) were inoculated into 30 mL of apple juice (pH 3.6,  $12 \pm 0.1$  °Brix) with initial spore concentration around  $10^5$  CFU/mL and exposed to UV- C light intensity ( $36 \text{ W/m}^2$ ) by the exposure times (0, 1, 2, 4, 7, 10, 15, 20, 30 min). Weibull and Log-linear plus tail model were fitted to  $\log N/N_0$  versus UV- C dose curves and goodness-of fit of these models was investigated.

**Results:** The results showed that UV-C radiation can inactivate *B. fulva* and *B. nivea* ascospores in apple juice. Log-linear plus tail model produced a better fit to the data with a higher coefficient of determination ( $R^2= 0.98; 0.95$ ) and lower root mean square error ( $RMSE= 0.314; 0.455$ ) values to *B. nivea* and *B. fulva* respectively. The Log-linear plus tail parameters were very close for both moulds ( $0.126 k_{max}$  and  $-4.75 N_{res}$  for *B. fulva*;  $0.113 k_{max}$  and  $-4.86 N_{res}$  for *B. nivea*). Based on Log linear plus tail model obtained, after 10 min of UV exposure ( $36 \text{ W/m}^2$ ) at a UV-C dose of  $21.6 \text{ kJ/m}^2$ , 4.4 log reduction was obtained for *B. nivea* and *B. fulva*.

**Conclusion and Relevance:** *B. fulva* and *B. nivea* ascospores had similar resistance to UV-C light. Models to describe the influence of UV-C dose on ascospore inactivation, for both moulds, were obtained. As a non-thermal technology, UV-C light is a promising treatment with great impact on the inactivation of heat-resistant fungi and could be used for the pasteurisation of apple juice.

**Keywords:** Ultraviolet light (UV-C); *Byssochlamys nivea*; *Byssochlamys fulva*; predictive mycology; apple juice

## **104: GROWTH OF *LISTERIA MONOCYTOGENES* AND *SALMONELLA* spp. ON READY TO EAT FRESH-CUT LETTUCE: MICROBIOLOGICAL COUNTS AND COMBASE PREDICTOR**

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**Introduction:** Consumption of fresh produce has been recognized as an important vehicle for the transmission of human pathogens. The consumption of these foods has been increasing among consumers who prefer healthy diets and provide an appropriate meal for the current lifestyles. Consumption of pre-washed leafy green salads has been increasing in parallel with consumer demand for convenient foods. In spite of their healthy and convenient aspects, microbiological safety should be a concern as growth of pathogens may occur during refrigerated storage in particular at abuse temperature conditions often observed at household level.

**Methodology:** Individual packages of pre-washed lettuce salad were inoculated with a cocktail of *Salmonella* or *Listeria monocytogenes* strains. Counts of the pathogens were followed during storage at different temperatures simulating refrigeration temperature abuse conditions observed in domestic settings. Growth under the same conditions was predicted using the ComBase Predictor software.

**Results:** Growth of *Listeria monocytogenes* and *Salmonella* spp. was observed on salads stored at temperatures above 5.5°C and 7°C, respectively. Higher growth rates were determined when ComBase Predictor was used.

**Conclusion and Relevance:** *Listeria monocytogenes* and *Salmonella* spp. can grow in fresh-cut lettuce even at short abuse of refrigeration temperature during storage. ComBase Predictor can be a useful tool to estimate the risk of consumption of lettuce stored at abuse temperatures at household level.

**Keywords:** ComBase Predictor; *Salmonella* spp.; *Listeria monocytogenes*; fresh-cut lettuce

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