Robustness of a cross contamination model describing transfer of pathogens during grinding of meat

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[O01.01]

Modelling the survival probability of *Salmonella enterica* and enterohemorrhagic *Escherichia coli* in a single cell level under desiccation environment

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Salmonella enterica and enterohemorrhagic *Escherichia coli* can cause foodborne illness by a small number of cells. The survival probability of a single cell level of these pathogenic bacteria can be used to assess their risk of causing foodborne illness. In this study, we focused on low water activity (a_w) and/or dried foods as critical pathogen carriers. The objective of this study was to develop a predictive model that can estimate the probability of survival of a single cell level of these bacteria under desiccated environment. The survival of the *Salmonella enterica* serotypes Stanley, Typhimurium, Chester and Oranienburg as well as *E. coli* O111, O26 and O157 were assessed. The single cells of each pathogenic bacterium were prepared via a 10-fold dilution of the bacterial culture. We controlled that the number of cells in each sample followed a Poisson distribution with mean value 2 to minimize the ratio of zero count. Single cells of each serotype were placed in microdroplets (2 µl) of distilled water on a 96-well microplate and exposed to temperatures ranging from 5°C to 25°C. The prepared microplates were then dried in a drying chamber (ca. 9% RH). We confirmed the survival of the cells in each well by adding nutrient broth (100 µl) at arbitrary intervals to promote cell recovery. Survival/death data was successfully described by using logistic regression as a function of time and temperature indicating that a higher drying temperature accelerated cell death. The changes in survival probability showed that more than 90% of the cells died during the drying process; however, a small fraction survived longer period that indicated the variability of the bacterial cells. This predictive model for estimating the survival probability of a single cell after drying could play an important role in assessing the risk of low a_w foods.

Keywords: low water activity, survival probability, variability, logistic regression

[O01.02]

Modelling the effect of calcium propionate, pH and water activity on germination of single spores of Penicillium paneum isolated from wholemeal bread

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Introduction: Bread spoilage by moulds is still a serious economic problem for bakery industry. Belonging to *Roqueforti section*, *Penicillium paneum* is commonly associated with spoilage of breads mostly due to the high resistance to organic acids, such as propionic acid. Bread contamination by this mould occurs mainly through deposition of low number of spores on the product surface during cooling step. Germination of a single spore can be enough to consumer rejection by appearance of visible mycelium before shelf life ends. The aim of this study was to analyse the influence of combined effect of calcium propionate, pH and water activity (a_w) on germination delay of *P. paneum* through a single spore approach.

Methods: A complete factorial experiment was performed (triplicates). The combined effect of pH (5.1-5.7), preservative (0.25 and 0.41%) and a_w (0.93-0.96) at 25^0C was studied. Assessment of germination was conducted using a 12-wells plate containing Potato Dextrose Agar. pH and a_w were adjusted with lactic acid and glycerol. Germination of single spores was examined in an inverted microscope (Zeiss Axio Observer.Z1) connected to iXon-3 CSU-X1 (Yokogawa, Japan) camera. Pictures were analysed using ImageJ 1.48. Distribution of percentage of germinated spores was fitted using logistic function. Distribution of germination time of individual spores was fitted using software @Risk 5.7 (Palisade Corporation, EUA).

Results: 96 data of germination time of *P. paneum* were obtained. At a_w 0.94, 0.25% of preservative and pH 5.1, only 30% of spores germinated after 96 hours. For pHs 5.5 and 5.7, the mean germination time were 51 and 47 hours, respectively, without significant difference (t-test, $p<0,05$).

Discussion: A decrease of pH and a_w values can strongly inhibit the germination of *P. paneum* spores. These results will be useful in the development of robust formulations aiming at increasing the shelf life of wholemeal breads.

Keywords: single spore, germination time, shelf-life, bread

[O01.03]

Single-Cell analytics of food contaminating bacteria

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Time-lapse microscopy now enables detailed imaging data generation and monitoring of dynamic processes at the single-cell level. Mathematical model development is of growing interest in capturing and testing hypothesis regarding the dynamical behavior of biological systems in food contamination and systems microbiology. Modeling bacterial communities of S. Typhimurium forming biofilms relies on the efficient and accurate extraction of single-cell information from time-lapse microscopy data of growing colonies. However, the analysis of such "cell movies" is currently very time consuming and error prone since it is essentially performed by human-experts. Here, we address this important limitation in a multi-resolution, high throughput, image analysis framework.

We developed an end-to-end methodology for identifying accurately the boundaries of individual bacterial cells and tracking them frame-to-frame so as to construct the cells' genealogy (segmentation and lineage tree construction) even in large-size microbial communities where identifying the individual cell boundaries is very difficult. Our resulting automated analytics pipeline combines image processing and machine learning methods to extract and track single-cells precisely while also estimating their properties (morphology, expression, location) and storing them for further analysis.

Our platform has been tested and evaluated with several cell movies of S. Typhimurium produced by different labs. It achieves high F-measure score (above 95%) consistently. It can analyze different image modalities data (phase contrast, bright field, and fluorescent images) produced by optical or confocal microscopy. Our analytics pipeline is automated, computationally efficient and suitable for high throughput bacterial cell movies processing without any human intervention for correction or calibration.

Acknowledgment: This work was supported by the action THALIS-BIOFILMS, co□financed by EU (European Social Fund-ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)□Research Funding Program: THALES. Investing in knowledge society through the ESF.

Keywords: SINGLE-CELL, IMAGE PROCESSING, CELL TRACKING, S. TYPHIMURIUM

[O01.04]

Modeling the impact of sub-optimal temperature and pH on the variability of germination and growth recovery among sub-populations of heat-stressed Bacillus weihenstephanensis KBAB4 and Bacillus licheniformis Ad978 spores

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Heat-treatment is a major hurdle widely applied for inactivation of bacterial spores in foods. The aim of this study is to follow by flow cytometry the fate of spores after specific heat-treatments and recovery in sub-optimal conditions. Especially the acquisition of kinetics and proportion of spores becoming vegetative cells were studied. A mathematical model taking into account the impact of recovery conditions and the dynamic aspect of the process was proposed.

The spores of *Bacillus weihenstephanensis* KBAB4 and *Bacillus licheniformis* AD978 were obtained at optimal and suboptimal growth temperature. After heat-treatments allowing a ten-fold reduction, spore recovery in nutritive broth was investigated for different pHs and temperatures distributed along the growth domain (5-40°C for *B. weihenstephanensis*, 15-60°C for *B. licheniformis* and pH 4.0-8.0 for both). The germination and growth recovery were monitored over time using flow cytometry, taking into account cell size (Syto9 staining) and respiratory activity (CTC staining).

Different physiological stages were efficiently evidenced: refractive spores, germinated spores, outgrowing cells and vegetative cells. In optimal conditions, most cells evolved rapidly towards multiplication. While in suboptimal conditions slower recovery process was observed and a lower proportion of spores successfully emerged into vegetative cells. Nevertheless, a large proportion of spores are subjected to germination and outgrowth after a heat-treatment, even only a few are able to form a colony on agar plates. A primary model was developed to describe this process.

Monitoring spore recovery using flow cytometry is a powerful method, allowing an exploration among cell populations evolution. The number of analysed cells is over 200000, offering an accurate estimation of variability in individual cell development within populations. The developed mathematical model describes the dynamic aspects of heat-treated spore recovery and takes into account the heterogeneous distribution of spores in the different physiological stages observed after a heat-treatment and the impact of environmental conditions.

Keywords: Bacillus, Spore, Recovery, Flow cytometry

[O02.01]

Robustness of a cross contamination model describing transfer of pathogens during grinding of meat

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This study aimed to evaluate a previously published cross contamination model (Møller et al. 2012) for its capability of describing transfer of *Salmonella* spp. and *L. monocytogenes* during grinding of pork and beef cuts of varying sizes (averaging 50 – 324g) using varying numbers of pieces to be ground (10 – 100) in two grinder systems. Data from a total of 19 grinding trials were collected. Three different evaluation approaches were applied: *i*) an Acceptable Simulation Zone (ASZ) method compared observed transfer with simulated output from the proposed model, *ii*) each grinding trial was fitted to obtain parameter estimates for integration in a Quantitative Microbiological Risk Assessment model estimating risk of salmonellosis (Møller et al. 2015), and *iii*) the Total Transfer Potential (TTP) was calculated for each trial from the belonging fitted parameter estimates.

The ASZ approach showed that the Møller et al. (2012) model could only describe seven of the 19 trials to an acceptable extent (70 % of observed counts being in the zone of simulated counts ± 0.5 log-units). However, observed transfer curves were well-described when fitted to the model structure proposed by Møller et al. (2012) (RMSE-values between 1.08 and 2.09). Results indicated that grinding trials performed at different conditions, such as different sizes and numbers of meat pieces to be ground, cannot be accurately simulated from parameter estimates obtained in unlike grinding processes. Comparison of risk estimates of similar grinding trials revealed that risk attribution from grinding was mainly influenced by sharpness of grinder knife > specific grinder > grinding temperature. Finally, a positive correlation was observed between risk estimates and TTPs suggesting that risk attribution from grinding systems can be assessed solely from the fitted parameter estimates.

Møller, C.O. de A. et al. (2012): Journal of Applied Microbiology 112:90-98

Keywords: microbial transfer, foodborne pathogens, meat processing, model evaluation

[O02.02]

A model based on the assumption that the specific growth rate is normally distributed as a function of time to describe microbial growth under isothermal and non-isothermal temperature profiles

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A mathematical model was applied to describe the growth of Clostridium perfringens in meat products both in isothermal and dynamic temperature profiles for different datasets found in the literature (ten isothermal and four non-isothermal). The model consists in the assumption that specific growth rate follows a Gaussian distribution as a function of the time. Application of this procedure results, after the mathematical derivations, a mathematical expression including the error function. This hypothesis is based on the central limit theorem that establishes that an event caused by many variables with influences of similar magnitudes follows a normal distribution. The parameters included in the model are the duration of the lag phase, the maximum specific growth rate and the logarithmic inflection point, referred in the literature as LIP. The values generated by the model were, in most cases, compatible with those predicted by the Baranyi-Roberts growth model, which is widely used in the specialized literature. It was observed for some isothermal datasets (three) that the duration of the lag phase was underestimated in relation to the result predicted by the Baranyi-Roberts growth model. However, the model predicts adequately a lag phase that is sharply defined in the experimental growth curve. In general, the statistical indexes (accuracy factor and bias factor) provided similar results for both models. In what concerns dynamic temperature profiles, the equation provided good qualitative agreement with the four profiles studied. Further studies are necessary in order to study the mathematical properties and implications of the equation.

Keywords: specific growth rate, gaussian distribution, central limit theorem

[O02.03]

Assessing the effect of risk mitigation by concentration reduction: Linear regression vs. Process risk model

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Basically two approaches can be taken in quantitative microbial risk assessment. A ”data based” approach considers the available data first, and often applies statistical methods to relate them. Alternatively, a ”mechanistic” approach first describes the dynamics of growth, survival and cross-contamination based on production processes, before suitable data are identified and applied in the models.

In this study we compared the performance of these two approaches in a case study on *Campylobacter* in broilers. We assessed the effect of reducing the *Campylobacter* concentration in the intestinal content of broilers on human risk of campylobacteriosis from consumption of broiler meat. For the “data based” approach, we used linear regression to describe the observed relation between concentrations in the caeca of the birds and the concentration on the skin after processing in Denmark. For the “mechanistic” approach, we applied a published process risk model describing the dynamics of cross-contamination and survival of *Campylobacter* during slaughter and processing. Finally, for both methods, a separate model was used to assess the human health risk on the basis of the concentration on the skin.

Results show that, roughly, these two different approaches give comparable results. However, both are also associated with large uncertainty and depend on the data sets used. Interestingly, when the process risk model is used to simulate the skin concentration data and predict the outcome of the linear regression analysis, both the variability in the data and the slope of regression line can be reproduced quite well.

The analysis shows that none of the approaches provides a simple predictive relation between reduction of concentration and risk reduction that can be generically applied. Hence it seems that the uncertainty associated to risk assessments is not a consequence of the method, but of the complexity of microbial dynamics in the processing environment.

Keywords: risk assessment, Campylobacter, broiler processing, cross contamination

[O02.04]

Variation in inactivation of Salmonella during carcass decontamination impacts the risk for consumers

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Whole carcass decontamination during slaughter has been considered as a control measure to reduce pathogen concentrations on meat. Its effect is usually measured in terms of the mean log reduction in concentration, although variation in the reduction may also contribute to the overall impact of decontamination. This study focuses on the relative contribution of mean and variation for the effect of decontamination during slaughter, expressed in terms of human health risk.

A stochastic risk model is developed to assess the potential effects of pig carcass decontamination at the end of slaughter on the risk of salmonellosis for Danish consumers. *Salmonella* concentrations on carcasses are represented by a lognormal distribution. Decontamination scenarios are represented by gamma distributions with different means and standard deviations, with values inspired on experimental data of real decontamination methods applied to pork.

Results show that the variation of decontamination has a relevant effect on risk reduction for the consumer: a larger variation results in a lower risk reduction. This effect is particularly evident for methods with lower mean reductions (≤ 2.5 $\log_{10}$). The impact of variation is also affected by the number of *Salmonella* bacteria contaminating the carcass. The higher the mean and standard deviation of *Salmonella* concentrations, the more relevant it is to account for the variation of decontamination, even if the mean effect is high.

We conclude that for decontamination methods with an overall mean reduction effect of 1 to 2 $\log_{10}$, if the variation is large, the final effect of decontamination can be considerably smaller than expected on the basis of the mean only and efforts should be put in place to reduce the variation of the method. However, when a treatment of high mean reduction (> 2.5 $\log_{10}$) is used, the impact of variation becomes smaller and may be negligible.

Keywords: carcass decontamination, log reduction, QMRA, consumer risk

[O03.01]

Effect of food intrinsic complexity on *Listeria monocytogenes* growth in/on vacuum-packed meat-based model systems at suboptimal temperatures

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Variations in intrinsic complexity ((micro)structure, composition and physicochemical characteristics) of foods influence microbial growth. The most recent targets focus on the investigation of the effect of food (micro)structure by comparing planktonic growth in liquid (microbiological) media with colonial growth in/on solid-like systems or on real food surfaces. However, foods are not only liquids or solids; they can also be emulsions or gelled emulsions with complex intrinsic characteristics.

In this study, *Listeria monocytogenes* growth was studied in/on the whole spectrum of food (micro)structure, in terms of food (model) systems with variable (micro)structural complexity, composition and physicochemical characteristics. The targeted (micro)structures were: i) liquids, ii) aqueous gels, iii) emulsions, iv) gelled emulsions and v) canned meat, which is a real food product and is classified as gelled emulsion. The composition and physicochemical characteristics of canned meat and gelled emulsions targeted real Frankfurters. All systems were vacuum packed and incubated at 4, 8 and 12°C. The most appropriate protocol for the preparation of the model systems was developed. The pH, water activity and resistance to penetration of the model systems were characterised.

Results indicated that *L. monocytogenes* grows faster on canned meat, followed by liquids, aqueous gels, emulsions and gelled emulsions, possibly due to the different source of protein and processing of the canned meat. Additionally, the canned meat exhibited the higher resistance to penetration than the other two solid systems (aqueous gel and gelled emulsion). This study has demonstrated that food model systems might underestimate microbial growth in comparison to what happens in/on real foods. Therefore, they require validation prior to further use for data collection. Furthermore, a successful model system should consider all aspects of food intrinsic factors, e.g., compositional and physicochemical factors of the target food product, in order to be successful for the assurance of microbiological food safety.

Keywords: Listeria monocytogenes, food (micro)structure, model systems, vacuum

[O03.02]

3D individual based model for simultaneous growth and interaction of L. Monocytogenes and lactic acid bacteria

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Objectives

By interacting with pathogens, lactic acid bacteria (LAB) are able to contribute to food safety. By means of their lactic acid production which induces pH decrease, LAB influence the growth of pathogens. The aim of this study is to model and simulate lactic acid production, pH evolution, according to carbohydrate concentration in media, temperature, water activity and ratio of both population.

Methods

To address the challenges related to the interdisciplinary context of this study, we have used a method based on two modeling steps.

In virtuo modeling relied on the Virtual Reality principle. RéISCOP software aim is to interactively model and simulate complex dynamical systems (1). We built models of *Carnobacterium piscicola* and *Listeria monocytogenes* interacting on a petri dish (2,3). Bacteria shapes are 3D capsules that have mechanical interactions. Bacteria feed on glucose and divide themselves over the time, depending on the parameters of their local environment. *Carnobacteria* produce lactate. The substrate was modeled as a simple 3D discretized reaction-diffusion system in which glucose and lactate diffuse. We made use of Unity3D to handle the graphical user interface that made possible to see, experiment and modify the model during the simulation. This *in virtuo* model was a toolbox to design an individual-based model from the cardinal populational data and model (2,3).

In silico optimized predictive model: based on this *in virtuo* modeling, we used the Transprog C library (4), so as to develop an optimized and parallelized model. Simplifications were made to allow simulation of hundred millions of cells (spherical shapes, only one bacteria layer in colony). Our validation process includes theoretical and experimental phases. For a single colony growth, we compare individual-based results and validated populational results. Secondly, with regards to the growth of two colonies in competition, we compare individual based models and experimental data.

Results

The first result of this study is the development of two softwares that can be easily re-used for other studies (we are trying 5). We have a virtual lab in which non-IT biologists can quite easily design models without coding. Furthermore, we have an optimized software to simulate various bacteria on a whole petri dish (10^7 bacteria individuals in real time on an ordinary PC).

Regarding the results of modeling, we are, at this time, calibrating and validating the models for three single colonies growing on a substrate: listeria, lactis and Carnobacterium. Experimental data for validation are already produced.

Conclusions

Data for validation have been collected and will be discussed. Validations remain to be achieved.

Individual-based model was a modeling key to study competition between colonies. We would like to be able to study the 3D colony shape and we envisage to model more complex food substrate.

In this study, using “virtual lab” was a methodological key to make us (biologists, computer scientists and mathematicians) be able to understand each other. We hope that this approach allows us to offer biologists new modeling techniques like individual-based model.

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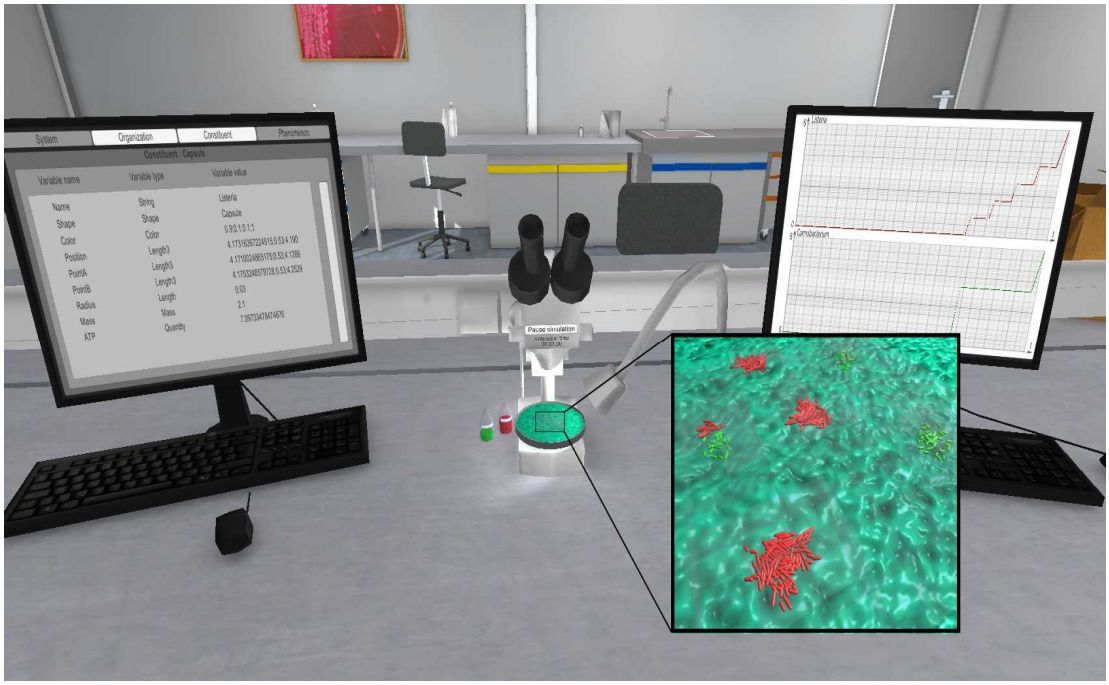

[O03.03]

Predictions of microbial thermal inactivation in solid foods: Isothermal and non-isothermal conditions

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Kinetic models that predict accurately microbial behaviour in real foods are an excellent tool to design adequate processing conditions.

This work focuses on the use of the Gompertz-inspired model to predict the thermal inactivation behaviour of microorganisms obtained in solid food products, validated for isothermal and non-isothermal conditions. Experiments were carried out in parsley, artificially inoculated with Listeria innocua. Three constant temperatures were imposed (52.5, 60.0 and 65.0 ºC) for isothermal experiments. For time-varying temperature conditions (from 21.0 to 65.5 ºC), a heating rate of 1.9 ºC/min was chosen for the first 24 minutes of the process. The temperature was then held at 65.5 ºC for the remaining thirteen minutes. Considering the temperature conditions, and parsley pH and water activity values, the microbial content was predicted and compared to experimental data. For the isothermal conditions tested, the predictive ability of the model was confined. The higher the temperature, the higher deviations observed (i.e. the model underestimates the inactivation behaviour). However, for the non-isothermal condition tested, the model predicted the microbial response accurately.

The results corroborate that microbial kinetic behaviour in “real” food surfaces differs to the one observed in broth. Consequently, caution should be taken when using the latter ones in food processing prediction. Nevertheless, and besides the required assessment when dealing with food systems, the model developed and expressed in terms of the most relevant variables studied (i.e. temperature, pH and water activity) allowed predictions that will certainly contribute to improvements of the preliminary designs of adequate thermal treatments.

Keywords: Experimental validation, Gompertz-inspired model, isothermal and non-isothermal conditions, solid foods

[O04.01]

Towards community driven food safety model repositories

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Transferring predictive microbial models from research into real world food manufacturing or risk assessment applications is still a challenge for members of the food safety modelling community. Such knowledge transfer could be facilitated if publicly available food safety model repositories would exist.

This research therefore aimed at identification of missing resources hampering the establishment of community driven food safety model repositories. Existing solutions in related scientific discipline like Systems Biology and Data Mining were analyzed.

On the basis of this analysis two factors could be identified which significantly promote the establishment of community driven model repositories: a standardized information exchange format for models and rules for model annotation. As a consequence the establishment of a Predictive Modelling in Food Markup Language (PMF-ML) is proposed and a prototypic implementation on the basis of SBML is provided. In addition a domain-specific extension of the MIRIAM guidelines for model annotation has been developed. In order to demonstrate the practicability of the proposed strategy, existing predictive models previously published in the scientific literature were re-implemented using an open source software tool called PMM-Lab. The models are made publicly available in an open Food Safety Model Repository called openFSMR.

This work illustrates that a standardized information exchange format for predictive microbial models can be established by adoption of resources from Systems Biology. Harmonized description and annotation of predictive models would also contribute to increased transparency and quality of food safety models.

Keywords: Model Repositlry, PMF-ML, standard

[O04.02]

An expert system for predicting the microbial growth and inactivation rates for different foodstuffs

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It is known that the growth of microorganisms is significantly influenced by both temperature (T) and water activity (aw). Furthermore, aw is related to the moisture content (MC) through sorption isotherms which can be described by equations such as GAB, BET and Modified Oswin. Once T and aw is known, equations can be used predict the growth or inactivation rate of different microorganisms.

United States Department of Agriculture (USDA) released the USDA National Nutrient Database data file to public that contains nutrient contents, including MC, of over 8000 different food materials and literature provides abundant of information on equations and their parameters that best describe the sorption isotherm of different foodstuffs. Furthermore, kinetic parameters, such as D- and z-values of different microorganisms are also available in literature.

A SQLite database has been compiled to incorporate all the above-mentioned parameters and an expert system was programmed to make decisions and select the correct equations based on the input from the user. A graphical user interface, similar to that of Microsoft Excel, was designed using the cross-platform wxWidgets library and the underlying code was developed in C++ language.

Using a partial differential equation solver (PDES), spatial and temporal variation of MC and T of food materials can be simulated under different storage/processing conditions. Therefore coupled with a PDES, the developed software can be used to determine the shelf-life or to design pasteurization/sterilization process of different foodstuffs.

Keywords: Expert System, Shelf life, Pasteurization, Software

[O04.03]

Dose-response modelling of Staphylococcal enterotoxins using outbreak data

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Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases and results from the ingestion of staphylococcal enterotoxins (SEs). Yet, except for a few volunteer trials and foodborne outbreaks, there is small amount of dose response information available for humans. The objective of this work was to establish a dose response based on the systematic investigations carried out during recent years in France.

Over the period 2010-2014, more than 60 SFP outbreaks involving staphylococcal enterotoxins, mainly from France, were microbiologically investigated. The enterotoxins were characterized as well as quantified. Attack rates, appearance times and natures of symptoms collected during epidemiological outbreak investigations were related to microbiological data.

The outbreaks collected focused on enterotoxins SEA, SEB, SEC, and SED. These SEs were present alone or in combination in the suspected foods. Distribution of appearance times of symptom and their natures (diarrhoea, abdominal pain, vomiting, nausea, and fever) were not influenced by the type of enterotoxins. The impact of the presence of single SE vs multiple SEs and of the type of food implicated were also investigated.

The US EPA benchmark dose (BMD) methodology was then used to establish dose response. Attack rates of SFP outbreaks were modelled as a function of ingested doses. Within all the available SFP outbreaks, after applying inclusion criteria (e.g. presence of a single toxin, well characterized attack rates), a BMD for SEA could be estimated.

Keywords: staphylococcal enterotoxins, dose-response, Outbreak investigation

[O04.04]

A meta-analytical approach to assess the inactivation of Salmonella spp., Escherichia coli O157:H7 and Listeria monocytogenes by different sanitizers during washing of fresh produce

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Washing of fresh produce with sanitizers have a great importance to reduce the microbial contamination which may impact on the safety and shelf-life of the final product. The aim of this study was to perform a meta-analysis of the effects of sanitizing treatments of fresh produce on *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

A total of 55 studies were found to report on the effect of sanitizers on the concentration of these pathogens in fresh produce, but the study was conducted with 40 works considering some selection criteria. In this study, two meta-analytical models have been built: *i)* a meta-analytical model aiming to assess the resistance of different pathogens against a sanitizer; *ii)* a meta-analytical model aiming to assess the effects of different sanitizers for the same treatment (constant time/temperature and sanitizer concentrations). Finally, a cluster analysis has been done to define and classify sanitizer based on their bactericidal effectiveness.

The results indicated that for most sanitizers, concentration, temperature and time have a direct effect on the microbial log-reduction. For some sanitizers, we identified a quadratic effect of temperature and differences among pathogens in their resistance to sanitizers. Overall, *L. monocytogenes* had a lower intercept, meaning that it may be more resistant, while *Salmonella* had a higher intercept, meaning that it tends to be the most susceptible of the three pathogens studied. It has been found that sanitization of leafy vegetables was less effective than other fresh produce. A dendogram built indicated the presence of four clusters of sanitizers according to their bactericidal efficacy. The results reported were obtained from a combination of data from 40 primary studies.

This seems to be an important achievement for advancing the global understanding of the effectiveness of sanitizers for microbial safety of fresh produce.

Keywords: Meta-analysis, Fresh-produce, Sanitizers, Pathogens

[O04.05]

On the assessment of meat products quality/contamination via non-invasive techniques: Estimation of 3-D multi-spectra spatiotemporal fingerprints

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To implement Process Analytical Technology (PAT) in the Food Industry, image processing and pattern recognition can be used for estimation and even prediction of food microbial quality. A novel methodology for food quality/contamination assessment in a way of increased objectivity, data reproducibility, low cost and faster information extraction is presented. Additionally the developed workflow solely relies on the data, i.e. no human intervention. First, a developed method of automated multispectral image analysis takes place, filling the gap in the area of food science in terms of unbiased information extraction from surface chemistry sensors. As a result we get the multispectral information of meat fillet samples excluding the surrounding, fat and connective tissues areas. Then, and after standard normal variate normalization, unsupervised classification is applied. Having in mind that spectra clusters are not necessarily spherical or ellipsoids and also some outliers will be present, we employed density based spatial clustering. We modified the algorithm so as to perform in an unsupervised manner in selecting the “natural” number of clusters. Applying the aforementioned method to a dataset consisting of a sterile subset (steady Total Viable Count- TVC from 1 to 2 log) and on contaminated subset (>2 to ~8 log TVC), monitoring them along time (in order to incorporate more variation), we believe we are then able to identify different characteristic classes of spectra. Those classes are then combined/compared, in order to exclude overlapping classes and get the non-overlapping ones. So, at the end of the proposed workflow, we detect/define “fingerprints” that concerns only the microbial contamination, while time related information is excluded. Additionally, we have the contamination’s spatial and spectral information evolving in time. The developed methodology is a novelty towards non-invasive quality assessment of food products from surface chemistry sensors.

This work has been supported by the project “Intelligent multi-sensor system for meat analysis-iMeatSense 550” co-financed by the European Union (European Social Fund–ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARISTEIA-I.

Keywords: Spoilage, surface chemistry, microbial contamination, no-destructive

[O04.06]

'Blown pack' Probabilistic modeling for C.algidicarnis and C.estertheticum under the effects of storage temperature, vacuum level and package heat shrink temperature

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This research aimed to model the 'blown pack' probability (BPP) caused by spores of *C.estertheticum* DSM8809 and *C.algidicarnis,* as a function of vacuum packaging control variables: storage temperature(ST), vacuum level(VL) and packaging heat shrink temperature(HST). Initially, spore suspensions were produced, standardized (~10^5spores/mL), and then they were inoculated, individually, on the surface of disinfected (100°C/2min) tenderloin portions beef (10x5x2cm) samples, to final level of 10^2spores/cm^2. After inoculum drying, meat portions were placed in individual sterile vacuum bags (BB2620), and vacuum pressured (VP) at 6 and 9mBar (Microvac, Selovac). Vacuum packs were shrunk by dipping in a water bath at 83 or 87°C/3s. Treated samples were stored at -2, 2, 4 and 15°C, in triplicate. Uninoculated control samples (32) for each condition/microorganism were processed in parallel with 96 test samples. Packs were daily visually monitored for up to 90 days for the presence of gas using method adapted from Boerema *et al*. (2007). Data treatment (gas/no-gas) was performed using the logistic distribution, selected according to Akaike Information Criteria (AIC), by JMP PRO 11 (SAS). The BPP model for *C.algidicarnis* was: $BPP_{C.algidicarnis}$=(4.11+0.365334)-0.161115(ST-4.75)-0.132804(VL-7.5)+0.099603(HST-85)+0,292096 – Eq.1. For this microorganism, storage temperature and vacuum level has a negative effect on BPP, while HST has a positive effect probably due to spore activation. For *C.estertheticum* model was: $BPP_{C.estertheticum}$=4.11+0.017845(ST-4.75)+0.147536(VL-7.5)-0.147718(HST-85)+0.292096 – Eq.2. By eq.2, storage temperature and vacuum level has a positive effect on BPP, and HST has a negative effect. For *C.algidicarnis,* model had accuracy of 93.75% and for *C.estertherticum,* accuracy was 81.25%. From model (1) the lower BPP for *C.algidicarnis* was 9% at: 3°C/6mBar/87°C; and for *C.estertheticum,* model (2), was 55% at 15°C/9mBar/83°C. For both microorganisms, tested variables were unable to eliminate the risk of blown package spoilage, probably because with 10^2spores/cm^2 tested hurdles lose their efficiency, still the spore levels in meat before vacuum must be controlled.

Keywords: psychrothrophic Clostridium, vacuum pack red meat, probabilistic model, 'blown pack'

[O05.01]

A systems level approach to potentiate the action of isothiazolinones against *Pseudomonas aeruginosa* and minimize biofilm formation

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Introduction. Recent developments in metabolic modelling enable us to better understand cell metabolism, thereby enabling us to find the most appropriate preservation strategies. Isothiazolinones are microbicides widely used as preservatives in water systems as well as in personal care and household products. In this work, we aimed to identify molecular targets that may potentiate the microbicide action against *Pseudomonas aeruginosa,* while minimizing biofilm formation.

Materials and Methods. A genome-scale model of *P. aeruginosa* metabolism under action of isothiazolinones was developed. Availability of carbon sources and amino-acids were defined to mimic water environments. Artificial Centre Hit and Run (ACHR) sampling was applied to determine distribution of fluxes through the metabolic network in absence of microbicide. To simulate the action of sub-inhibitory concentrations of isothiazolinones, the upper bound of distribution of fluxes affected by the microbicide were reduced to a fraction of their mean value. Using Flux Balance Analysis (FBA), simulations of gene deletions were then performed *in silico* to identify individual and pairs of genes that could be targeted to achieve complete growth inhibition. Finally, the framework of Xu *et al.* (2013) [*PLoS One 8:e57050*] was implemented to assess whether partial inhibition of these targets might activate biofilm formation.

Results. Single gene deletion analysis allowed the identification of 37 genes whose deletion could have moderate synergistic effects with isothiazolinones. Double gene deletions were simulated to identify combinations of these genes that are potentially lethal when removed in pairs. The list of identified targets was ranked, based upon the potential capability of their mutants to form biofilms.

Discussion. This case study demonstrates the potential of mechanistic models to optimise and design novel preservative strategies. Incorporating specific information on microbicide mode of action in the model allowed us to identify some potential targets that would not have been detected otherwise.

Keywords: Flux Balance Analysis, systems biology, Pseudomonas aeruginosa

[O05.02]

The incorporation of a noncomplex metabolic model for Escherichia coli based on systems biology concepts in an individual-based model for the simulation of biofilm dynamics

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Introduction: In natural environments, microorganisms normally grow on surfaces, e.g., as biofilms, rather than planktonically as freely suspended cells. In biofilms, cells exhibit a wide range of physiological states, as a result of microscale chemical gradients, adaptation to local environmental conditions, stochastic gene expression, and genotypic variation due to mutation and selection[1]. For facultative anaerobic species, like *E. coli*, biofilm growth is particularly dependent on oxygen availability, with the absence of biofilm formation at anaerobic conditions[2]. To model oxygen gradients and heterogeneous cellular behavior in biofilms, the cell is considered as basic unit in a spatially-explicit individual-based model (IbM).

Methods: Based on systems biology concepts, a computationally-efficient metabolic model has been developed for *E. coli*, covering all metabolic regimes from aerobic respiration to anaerobic fermentation. This metabolic model links the uptake of glucose and oxygen with the production of biomass and the excretion of weak acid metabolic products. The growth-inhibiting effects of low environmental pH values and weak acid cell products have been taken into account. The developed metabolic model is further incorporated in an IbM to simulate particular aerobic and anaerobic cellular behavior in *E. coli* biofilms.

Results: With the developed individual-based model, oxygen gradients in *E. coli* biofilms and their effect on cellular behavior can be modeled. Over time, the bottom layers of the biofilm get oxygen-depleted leading to detachment of the biofilm cells from the surface.

Discussion: The relevance of research on biofilms is evident from their negative impact in medical, industrial and environmental contexts. In this work, an IbM is applied to specifically simulate the influence of oxygen gradients on metabolic differentiation in biofilms. This IbM provides the possibility to incorporate genotypic differences between cells and intercellular communication, which is the direction of our further research.

References:

[1]*Nat Rev Microbiol. 2008 Mar;6(3):199-210.*

[2]*Res Microbiol. 2004 Sep;155(7):514-21.*

Keywords: Individual-based modeling, Single cell modeling, Metabolic model, Biofilms

[O05.03]

A dynamic network to describe the adaptation of foodborne pathogens to osmotic stress: Application to Escherichia coli and comparison with Salmonella

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In predictive microbiology, the growth rate of food-borne pathogens is routinely predicted as a function of the environment. However, when the cells are not dividing but "surviving" or just adapting, as for example during the lag time, it is difficult to quantify their physiological state. Here, we define dynamic subnetworks of the "potential" regulatory network of the model organism *E.coli* K12 during the lag time to describe the adaptation process. The methodology leads to a new representation of the cells' physiological state.

The regulatory network of the model organism *E. coli* K12 (see, for example, RegulonDB, http://www.ccg.unam.mx/en/projects/collado/regulondb) includes all the known possible interactions between genes or operons and transcription factors. It results in a potential network, with specific manifestations for the actual environment. To study the effect of the environment, we measured the gene expression profiles of *E. coli*, using micro-arrays, during the lag time (6 time points) in a minimal medium, at 37°C at 2, 4.5, 5 and 5.5% NaCl (see Metris *et al.*, 2014 Appl. Environ. Microbiol. 80(15):4745). We embedded the data in the potential network and followed the cells' temporal adaptation to osmotic stress via the dynamic changes in the subnetworks. To compare with *Salmonella* Typhimurium, we inferred a potential regulatory network (Metris *et al.*, submitted) in which we embedded Salmonella temporal gene expression for NaCl concentrations of 2, 3.5, 4.5 and 5%.

We conclude that, in the same way as the growth rate is characteristic of environmental conditions, sub-networks constructed from gene expression data are useful representations of the physiological state of bacteria. In this case, gene expression profiles were embedded into a regulatory network, however other –omics data can be utilised in a similar fashion.

Keywords: Dynamic Network Analysis, Salmonella Typhimurium, Physiological state, Osmotic stress

[O05.04]

Whole genome sequencing for microbial risk sssessment of foodborne pathogens

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The potential for using whole genome sequencing (WGS) data in microbiological risk assessment (MRA) has been discussed on several occasions since the beginning of this century. The major challenge is to develop a common methodology that bridges the gap between the wealth of data generated by WGS and practical food safety applications. The difficulty is due to the non-trivial problem of mapping microbial information consisting of thousands of loci onto a probabilistic scale for risks.

We will present a first approach in methodology development for the application of WGS data in MRA supported by some practical examples. We use available data from a highly reproducible standardized *in vitro* system (*i.e.* simulated gastrointestinal tract) as a measure for the virulence of STEC (O157) and *Salmonella* (serovar Typhimurium/Enteritidis versus exotic serovars isolated from imported foods). Both genotype-phenotype matching and comparative genomics of strains are performed to identify genetic elements that differentiate (groups of) strains in terms of virulence.

This application revealed practical implications when using SNP data for MRA. These can be summarized by considering the following main issues: optimal sampling from the genetic space, correction for population structure, quantification and calibration, reproducibility, links with epidemiological data, anchoring and integration for the translation of molecular studies to human health risk.

The differentiation of high virulent types from less virulent types will provide a basis for more specific risk assessment, targeted risk based monitoring and science-based enforcement.

Reference:

Pielaat, A., Boer, M.P., Wijnands, L.M., van Hoek, A.H.A.M., Bouw, E., Barker, G.C., Teunis, P.F.M., Aarts, H.J.M, and Franz, E. (2015) First step in using Molecular Data for Microbial Food Safety Risk Assessment; Hazard identification of E. coli O157:H7 by coupling genomic data with in vitro adherence to human epthelial cells. Int. J. of Food Micro. DOI: 10.1016/j.ijfoodmicro.2015.04.009

Keywords: MRA, GWAS, STEC, Salmonella

[O05.06]

Ecology from Farm to Fork of microbial drug Resistance and Transmission (EFFORT) - quantification of transmission of antimicrobial resistance from animals to humans

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Ecology from Farm to Fork Of microbial drug Resistance and Transmission (EFFORT) is a five-year EU FP7 project which focuses on understanding the eco-epidemiology of antimicrobial resistance (AMR) from animal origin and its transmission to humans. The EFFORT trans-disciplinary consortium is made up of 20 partners from 10 European countries. The scientific research is divided in 8 work packages: 1) integrated evidence base, 2) molecular ecology and epidemiology, 3) ecology and transfer of resistance, 4) integrated epidemiological analysis, 5) resistance and usage, 6) intervention studies, 7) exposure assessment and 8) economic impact analysis. EFFORT's aim is to provide scientific evidence and high quality data to inform decision makers and the scientific community about the consequences of AMR in the food chain.

In work package 7, a comparative exposure assessment will be developed to estimate the relative importance of different food and animal transmission routes for human exposure to AMR determinants in the overall population. Furthermore, a source-attribution model will be developed to estimate the relative importance of food, animal-contact and environmental sources of AMR determinants for humans with occupational exposure at farm and slaughterhouse level. The estimates for occupational exposure will be integrated in the global comparative assessment. Last, the global model will be refined by integrating estimates of transfer rates of AMR determinants from commensals to pathogenic microorganisms in the human gut, in order to estimate risk of infection through the major transmission routes.

By supporting political decisions and the prioritization of risk management options along the food chain, the results of EFFORT will contribute to limiting human exposure to antimicrobial resistance. Furthermore, by producing and using as input sequencing data of single isolates and of whole bacterial communities, the project will urge the use of metagenomics data in exposure assessment and source-attribution modelling.

Keywords: EFFORT, Antimicrobial resistance transmission, Metagenomics, Exposure assessment

[O06.01]

Low temperature and reduced water activity during conidiation shorten germination time of Penicillium species

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While there is an increased knowledge on the effects of abiotic factors on fungal spore germination using spores produced under optimal conditions, little is known on the effects of such factors when spores are produced under sub-optimal conditions. In this context, using a monofactorial experimental design, we investigated the effects of temperature, pH and water activity (a_w) applied during conidiation, on the germination time of *Penicillium expansum* and *Penicillium roqueforti*.

Three temperature (5, 20 and 27°C), pH (2.5, 4.2 and 8) and a_w (0.90, 0.98 and 0.99) values were applied for spore production. Subsequently, for each factor and at each of their three levels, the germination kinetics of conidia were established by phase-contrast microscopy on agar medium. Germination time (time to 50% germination) and variance of germination rate (reflecting heterogeneity among spore populations) were estimated by fitting primary asymmetric models for germination.

For *P. roqueforti* but not for *P. expansum*, germination time at 5°C was significantly shorter for conidia produced at 5°C than for conidia produced at 20°C and 27°C (70.94 ± 0.67, 104.90 ± 0.95 and 122.47 ± 1.70 hours respectively). Moreover, conidial populations produced at 27°C were more heterogeneous than populations produced at other temperatures. Both species exhibited significant shorter germination times at a_w 0.90 when conidia were produced at a_w 0.90 as compared to conidia produced at a_w 0.98 or 0.99. Low temperature and reduced a_w exacerbated heterogeneity among conidial populations, regardless of the tested conidiation conditions.

In conclusion, as shown in the present study, fungal germination may be misestimated using conidia produced under optimal conditions since conidia encountered in industrial environment are rarely produced under such conditions. It may be of interest to consider the conditions for conidiation in the gamma model concept to improve the efficiency of predictive models.

Keywords: Fungal spore germination, Modelling, Penicillium, Conidiation conditions

[O06.02]

Growth abilities of Mucor spp. in dairy environment: From experimental data to predictive modeling

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The *Mucor* genus includes ubiquitous species regularly encountered as contaminants of foodstuffs where they induce undesirable effects including off-flavours, anomalous textures or discolorations. In the cheesemaking context, some *Mucor* spp. are used as technological microorganisms contributing to the texture and the sensory characteristics of cheeses such as Tommes while, on other cheeses, they can cause spoilage. To date, growth abilities but also positive and negative impacts of *Mucor* spp. on cheese are poorly known.

The aim of this study was to investigate the behavior of contaminant and technological *Mucor* strains under different physicochemical conditions encountered in the dairy industry.

The impact of abiotic factors (temperature: 0 to 50 °C; water activity: 0.84 to 0.99; pH 1.8 to 13.6) on the growth of strains pertaining to different species commonly encountered in cheeses (*M. circinelloides, M. racemosus, M. lanceolatus, M. fuscus, M. spinosus, M. brunneogriseus*), as well as of a plant endophyte *(M. endophyticus*, as an outgroup*)*, were determined on synthetic Potato Dextrose Agar medium (PDA). Assessment of radial growth kinetics including a lag phase followed by a constant radial growth of the mycelium allowed to estimate cardinal values for each species and abiotic factor. These data were consequently used to design and validate a model to predict the growth rate of the different species on Cheese Agar medium and actual cheeses (Tomme de Savoie and goat cheese). Comparison of experimental and modeling growth rates on cheese matrices showed that our model is relevant for process optimization in the cheese industry. Optimal growth rate values estimated on cheese matrices were generally lower than those obtained on PDA except for technological strains which seem to be adapted to the cheese environment. For some conditions exhibiting major phenotypic differences, a proteomic approach is currently carried out to identify proteins potentially involved in this adaptation.

Keywords: Mucor, Cheese, Predictive model, Alteration and technological flora

[O06.03]

Growth-no-growth boundaries of Penicillium paneum on multigrain wholemeal bread as a function of pH, water activity, calcium propionate and temperature

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Introduction: The increase of shelf life of wholemeal bread is a challenge for bakery industry. Although many alternatives have been suggested, as inactivation methods for contaminants, a viable and cheap alternative for most of industries is to develop a robust formulation. The aim of this study was to determine growth boundaries of *Penicillium paneum*, a representative spoilage mould, in wholemeal bread through a probabilistic modelling approach.

Methods: A full factorial design was built using multigrain wholemeal bread slices (6g) formulated with different levels of pH (5.1-5.5), water activity (a_w) (0.93-0.96) and calcium propionate (0.25, 0.33 and 0.41% per weight basis). For each treatment, six bread slices were placed in Petri dishes containing appropriate amount of glycerol solutions with identical water activity values of bread. The bread slices were inoculated with 5μL of a spore suspension of *P. paneum* (1-5 $\times 10^5$ spores/mL) and further sealed with Parafilm®. The inoculated bread slices were then incubated at 20^0C, 25^0C and 30^0C and monitored daily for up to 30 days until the appearance of visible mycelium. The results were converted as growth probability (0-1) with 0, for no growth and 1 for growth (>3mm). The data were fitted to logistic regression as secondary model.

Results: It has been found that the parameters a_w and pH had significant effect on growth of *P. paneum* in wholemeal bread at all temperature values studied. The combined effect of lower pH value (5.1) and minimum concentration of preservative (0.25%) resulted in a delay of growth for all temperature and a_w values studied.

Discussion: The growth boundaries of *P. paneum* obtained from probabilistic models can be used as a tool for bakery industry to develop a robust formulation and increase shelf life of breads.

Keywords: multigrain wholemeal bread, mould, probabilistic modelling, shelf life

[O06.04]

Influence of temperature, water activity, pH and organic acids on the germination time of Penicillium camemberti and Penicillium roqueforti

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Introduction : *Penicillium camemberti* and *Penicillium roqueforti* are two species that are widely used in the cheese industry. They are used as starters to prevent from spoilage by other organisms and to produce aromatic compounds. It is crucial to control conidial germination of these species.

Methods : Predictive modelling was used to assess the effects of environmental factors on the germination time. Asymmetric and cardinal models with inflection were used as primary model for germination, and secondary model, respectively.

Results : The effects of temperature, water activity, and pH did not depend significantly, p=0.05, on the species. The minimum values were 0.83 a_w, -1.4/-0.2°C, and 2.84/2.91 pH, the optimum 0.99 a_w, 26.9°C and 5.52/5.68 pH, and the maximum 33.5°C and 13.8/14 pH. The effects of lactic acid, and propionic acid were assessed at pH 5.6, on Potato Dextrose Agar, at 25°C. Linear relationship between the reciprocal of the germination time and the concentration of the organic acids was exhibited for *P. camemberti*. Lactic acid at 1M could not inhibit completely germination of the two species. However, *P. camemberti* was less resistant to the two organic acids than the other species. At pH 5.6, the minimum inhibitory concentration, CMI, for propionic acid was evaluated by a new model compatible with the gamma-concept. MIC values were 0.197M (14.6 g/l), and 0.796 (59.0 g/l), for *P. camemberti* and *P. roqueforti*, respectively. By using the asymmetric model for germination, viability of the conidia was also determined. A decrease in the viability for the two species was observed with increasing the concentration of organic acids. At 0.18M propionic acid, 28.0% of *P. camemberti* conidia were viable. At 0.50M propionic acid, 58.7% of *P. roqueforti* conidia were viable, however some germinated conidia did not produce hyphae.

Conclusion: This study also demonstrated that predictive mycology tools are not restricted to food spoilage moulds but can be extended to starters used in the food industry.

Keywords: Germination, Penicillium, Organic Acids, Environmental factors

[O09.01]

Adaptive response to acetic acid impacts on lag time distribution, growth /no-growth boundaries and inactivation rate of Bacillus weihenstephanensis

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Organic acids, and particularly acetic acid are extensively used in food formulation to prevent microbial development. This study aims at evaluating the impact of culturing conditions on growth and resistance to acetic acid exposure.

Bacillus weihenstephanensis KBAB4 cells were grown in BHI at 30°C either at pH 7.2 (non adapted cells) or pH 5.5 adding 14mM of acetic acid (adapted cells). End exponentially cells were used as an inoculum. Resistance was evaluated exposing adapted and non-adapted cells to different concentrations of acetic acid (from 0 mM to 50 mM acetic acid, pH 4.7, 30°C) in order to determine inactivation kinetics. While growth ability was evaluated exposing cells to sub-lethal concentration of acetic acid (14mM acetic acid, pH 5.5, 30°C) or to optimal conditions (pH 7.2, 30°C) to further quantify growth rate and the distribution of individual lag time using Bioscreen.

The bacterial resistance decreased with increasing acetic acid concentrations. For instance, a 3 log population reduction of non adapted cells was estimated at 25 hours and 2.5 hours, for 2mM and 10 mM acetic acid, respectively. As expected, adapted cells were more resistant than non adapted cells. At 10 mM acetic acid, 99% population reduction was observed after 1.5 hours of exposure for non adapted cells as compared to 31 hours for adapted cells. Furthermore, for adapted cells, the remaining population (1%) was able to grow in given conditions. Regarding growth ability, the mean lag time and the heterogeneity varied with the acetic acid concentration.

Adaptive response to acetic acid impacts on lag time distribution, growth/no-growth interface, and inactivation rate of *Bacillus weihenstephanensis*. The identification of biomarkers such as gene expression may be of interest to further qualify bacterial physiological state and integrate bacterial fitness and adaptive response into predictive modeling tools.

Keywords: acetic acid, bacillus weihenstephanensis, growth ability, bacterial resistance

[O09.02]

Modeling biofilm formation by Staphylococcus aureus challenged with sub-inhibitory concentrations of the essential oil from Origanum vulgare L.

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The ability of *S. aureus* to form biofilms enhances it survival in food-contact surfaces and food processing environments. Increasing concern about the safety of the traditional sanitizers has motivated research of natural antimicrobial compounds, such as *Origanum vulgare* L. essential oil (OVEO). There is a lack of information regarding the effect of OVEO on biofilm formation.

The objective of this study was to evaluate and model the effects of the exposure to the stress conditions imposed by sub-inhibitory concentrations of OVEO on biofilm formation in *S. aureus*.

Two *S. aureus* strains (LPM 11 and LPM 86) isolated from food-contact surfaces were growth in stainless steel surfaces immersed in meat broth containing OVEO (2.5, 1.25 and 0.62 µL/mL) during 72h at 35°C. Over a period of 15-days the planktonic and sessile cells in surfaces were assessed at intervals of 72 h using viable counts procedure. Controls were performed without OVEO. DMFit was used to model the effect of time on viable count over the 15-day period. Secondary models were developed using linear regression in Microsoft Excel.

A decrease ($p \leq 0.05$) in sessile cells of approximately 2 log CFU/mL and 1 log CFU/mL occurred when *S. aureus* growth in meat-broth containing OVEO at 2.5 µL/mL and 1.25 µL/mL, respectively, for both strains after 15-days. Exposure to OVEO at 0.62 µL/mL caused an increase ($p \leq 0.05$) of cells in biofilms (1 log CFU/mL) after the 10th day. Secondary linear models for the effect of OVEO concentration on rate of change of bacterial population were markedly improved ($r^2 > 0.97$), when controls were removed from the model ($r^2 < 0.8$).

This information will be value to predict the behavior of *S. aureus* biofilm producer on stainless steel surfaces in the presence of OVEO. The research also indicates the possible stimulatory effect of low OVEO concentrations on *S. aureus* biofilm formation.

Keywords: biofilm, stress condition, S. aureus, essential oils

[O09.03]

Modelling and validation of performance of Listeria monocytogenes wild type and stress resistant variants in simulated food chains

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The dynamic response of pathogens to environmental changes depends on the behaviour of individual cells within the population. Exposure of *L. monocytogenes* to different stress conditions has been shown to result in selection of a variety of stable resistant variants. This population diversity allows for growth and survival of the population under a wide range of environmental conditions with specific fitness and robustness parameters considered a trade-off; a variant may have an advantage under one condition, while this might be a disadvantage under other conditions. Therefore, the types of variants and their relative contributions within the WT population will depend on the environmental conditions encountered by the population. This study aims to evaluate the effect of environmental conditions on the composition of the *L. monocytogenes* populations and how dynamic conditions affect the fraction of stress resistant variants within the population. Growth parameters were obtained for WT and a set of eight acid resistant *L. monocytogenes* variants. A gamma model was used to estimate the growth behaviour under combined mild stress conditions (temperature, water activity and pH). Also a set of inactivation parameters was determined (heat and acid). This set of robustness and fitness parameters of WT and variants was used to model their performance in simulated food chains. Predictions were validated by qPCR in which WT and variant were distinguished from each other by specific primers, designed on an *rpsU* mutation in the variant. With this method, a variant fraction as low as 10^{-5} could be identified correctly in a WT population. This study provided more insight in the conditions which can select for variants, which is an important step in control of these stress resistant subpopulations in industrial settings. It also highlights the potential persistence of stress resistant variants in food processing environments and consequential impact on food safety.

Keywords: stress resistant variants, population dynamics, persistence, food chain modelling

[O09.04]

Assessing the capacity for survival, growth and acid adaptive response of Listeria monocytogenes during storage of various cheeses and subsequent simulated digestion

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Different physicochemical and microbiological characteristics of cheeses may affect *Listeria monocytogenes* potential to grow, survive, or exhibit an acid adaptive response during storage and digestion.

We aimed to estimate: i) the ability of various cheeses to support growth of *L. monocytogenes* during storage and ii) the impact of *L. monocytogenes* habituation on cheeses surfaces on the subsequent acid resistance during simulated digestion.

Cream (Mascarpone, Cottage), soft (Mozzarella, Camembert, Mastelo, Anthotyros, Manouri, Ricotta), and semi-hard (Halloumi, Gouda, Edam) cheeses were purchased and inoculated with 100 CFU/cm^2 or g of *L. monocytogenes* (vacuum or aerobic conditions; at 7°C)(*n=4*). The impact of indigenous technological or spoilage microbiota on pathogen growth was evaluated by purchasing cheese samples throughout different time points of their shelf-life. PH and a_w were monitored. Growth rates of *L. monocytogenes* (DMFit) were compared to expected results from existed predictive models (Combase, GroPin). Pathogen survivors were enumerated during exposure to simulated gastric fluid (SGF)(pH 1.5; HCl; 60 min) using thin agar layer method, while presence/absence was investigated through enrichment in cases where population levels were <10 CFU/cm^2 or g (enumeration limit)(*n=6*).

Mascarpone, Mozzarella, Camembert, Ricotta, and Halloumi supported *L. monocytogenes* growth by increasing 0.5-0.8 log CFU/cm^2 or g per day, since low initial TVC levels (1.8-3.8 log CFU/cm^2 or g) and high pH/a_w values (6.23-6.66/0.965-0.993) were recorded. On Cottage, Anthotyros, Manouri, Mastelo, Edam, and Gouda, the pathogen survived close to the inoculation level due to the high competition (6.8-7.1 log CFU/cm^2 or g) and/or low pH/a_w values (5.00-5.71/0.952-0.967). *L. monocytogenes* growth was significantly inhibited ($p<0.05$) on batches purchased close to expiration date (high initial TVC levels) compared to those close to production date, regardless of cheese type. The pathogen showed increased tolerance during storage of cheeses which supported growth, while on those that only survival was recorded, presence of *L. monocytogenes* (16-33%) was verified after 60 min exposure to SGF.

Such findings may provide useful evidence for assessing the risk posed by various cheeses types in relation to their compliance with food safety regulations.

Keywords: L. monocytogenes, cheeses, potential growth, simulated digestion

[O09.05]

A kinetical model to describe the growth and the sporulation of Bacillus subtilis

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Spore-forming bacteria represent a great deal for food safety. They can differentiate into spores which are able to resist and survive to different kinds of treatments applied in the food industry. Predictive microbiology has proved its efficiency to prevent the vegetative cell growth, but sporulation models are sparsely used and generally are developed independently of the vegetative cell growth. However, the sporulation process is closely related to growth but no model allowing to deduce a sporulation kinetic from a vegetative growth kinetic has been developed yet.

The purpose of this study is to suggest a growth and sporulation kinetic model allowing to label the efficiency of the sporulation process based on already available models describing the vegetative cell growth (e.g. growth rate, lag time and cardinal values).

Bacillus subtilis BSB1 was used in this study as it is derived from the well-studied strain 168. Growth-sporulation kinetics were performed in brain heart infusion supplemented with sporulation salts, at pH 7.0, under 100 rpm agitation, with an initial inoculum of 3.0 log10 (CFU/mL). Total cells concentrations were enumerated by plating on agar medium and spores were enumerated by plating after a 10 minutes treatment at 80°C of the total culture. Kinetics were performed at 18°C, 37°C and 45°C. The goodness of fit of the model allowed to evaluate the effect of temperature on sporulation behavior of *B. subtilis*.

A mathematical model was proposed to describe both the vegetative cell growth kinetic and the spore formation. This model integrates sporulation parameters with a biological meaning: the probability of a vegetative cell to sporulate, the time for sporulation initiation and the sporulation rate.

This new kinetic model allows to predict correctly sporulation behavior of *B. subtilis* and thus constitutes a good tool to prevent spore formation according to environmental factors.

Keywords: kinetic modeling, sporulation, Bacillus subtilis, temperature

[O10.01]

Modelling the microbiological risk of Salmonella spp. and Listeria monocytogenes in potato and chicken salad

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Different formulations of deli salads aim to satisfy consumer demands for products of mild acidity, but may also raise safety concerns due to potential survival of enteric pathogens or growth of *Listeria monocytogenes*. We aimed to: (i) model the survival of *Salmonella* spp. in mayonnaise (cream)-based chicken and potato salad and, (ii) describe the growth/no growth interface of *Listeria monocytogenes* in chicken salad, as affected by the initial pH of the cream salad, the concentration of the chicken and potato particulates, the type of the acidulant and the presence or absence of preservatives. Cream salads were prepared with initial pH of 3.6, 3.9, 4.1 and 4.4, adjusted with acetic or lactic acid, with or without 0.3% sodium sorbate. Pre-boiled potato (0.7 x 0.7 cm) or chicken (0.4 x 1.0 cm) particulates were added in 4 0%, 33.3%, 50% and 75% portions. The samples were inoculated (6 log CFU/g) with a five-strain composite of *Salmonella* spp. or *L. monocytogenes* and stored at 5^{o}C. Inactivation curves of *Salmonella* were fitted by the log linear model, to estimate the classical D value. A Bigelow-like model was used to describe the logD as a function of the initial pH of the cream salad and the concentration of the particulate. The growth probability (Logit*P*) of *L. monocytogenes* was determined by fitting the G/NG responses in the products after 30 days at 5^{o}C, to a second-order logistic regression model for P=0.5. The behaviour of both pathogens was predominantly affected by the equilibrium pH of the final products, resulting from mixing the acidified cream salad with the particulates. The addition of potato or chicken up to 75% increased the pH of the final products by 0.1-0.5 and 0.3-1.5, respectively, within 24 hours and further enhanced the survival of *Salmonella* and the growth of *L. monocytogenes*. Acetic acid exhibited higher antimicrobial properties than lactic acid, especially in the presence of preservative. The developed models may assist in the safety-by-design development of new formulations for products with mild acidity, without compromising their microbial safety.

Keywords: Salmonella, L. monocytogenes, deli salads

[O10.02]

How to decide processing standards for the processing of beef meat intended to be eaten raw

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In the earlier 2011, an enterohemorrhagic *Escherichia coli* (EHEC) outbreak was occurred due to the intake of contaminated raw beef dishes in Japan. As of the end of June 2011, over 160 patients were reported to be suffered from the foodborne infection, in which four patients were killed with development of HUS.

To ensure safety of raw beef dishes, Japan Ministry of Health, Labour, and Welfare (MHLW) had decided to argue appropriate standards based on the Food Sanitation Act. According to the literature-based means for the prevalence of EHEC associated with beef meats, new standards at the stage of meat slaughter and cutting has been established. This approach finally provided sampling plan that >25 samples (with 25g each) per meat block (considered as one rot) should be tested for the prevalence of *Enterobacteriaceae.*

To minimize the contamination risks at the processes from cutting to consumption, we also investigated the related hygienic practices and proposed to handle the *Enterobacteriaceae*-negative meat block under hygienic conditions, followed by packaging in hygienic air-tight bags and heat-processed by a method allowing that at least 1 cm subsurface of the meat sample to be held at >60ºC for at least 2 minutes or alternatives.

This microbiological criteria for processing of raw beef meat dishes has been thereafter evaluated by Japanese risk assessment organization, Food Safety Organization (FSO) and finally becoming a new criteria in the Food Sanitation Act on the beginning of October 2011.

In this presentation, we report the above flows mainly focusing an approach of microbiological criteria, and experimental results to evaluate the efficacy of heat-processing on the reduction of EHEC contamination in beef meats.

Keywords: EHEC, beef meat, microbiological criteria, FSO

[O10.03]

Understanding the risk of Listeria monocytogenes in retail delicatessens

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A discrete event model that mimics an operating delicatessen (deli) of a retail food store, tracks the *Listeria monocytogenes* (*Lm*) cells that may potentially be present in the environment and on food products and estimates the risk of listeriosis from consumption of ready-to-eat (RTE) foods commonly prepared and sold in those delis was described in Pouillot et al., 2015 (Journal of Food Protection, 78(1):134-45). This complex model integrates recent growth, inactivation and cross-contamination predictive microbiology models from the literature. In this study, the quantitative risk assessment (QRA) model is used to more fully evaluate factors and conditions that contribute to an increased risk of listeriosis from RTE foods prepared and sold in retail delis. The QRA model also serves as a "virtual laboratory" to evaluate the public health impact associated with changes in current retail deli practices and interventions. The results of this study suggest that reducing the risk of listeriosis associated with RTE foods prepared and served in retail delis is dependent upon *i*) the control of growth of *Lm* in RTE foods by formulating them with growth inhibitors during manufacturing or through better temperature control at retail, *ii*) the control of cross contamination during the routine operation of the retail deli, *iii*) the control of *Lm* contamination at its source in the deli, notably the control of contamination on incoming RTE products and the control of environmental contamination and niches in the retail environment, and *iv*) regular sanitation. This QRA also confirms the deli slicer as the primary site for cross contamination of RTE products at retail, illustrating the extent changes in slicer operations influence the safety of RTE foods prepared in delis. This innovative model improves our understanding of the complex factors that impact the public health risk associated with RTE products prepared in the retail deli.

Keywords: Risk assessment, Listeria monocytogenes, Cross Contamination, Discrete Event Model

[O10.04]

Process risk model for *Salmonella* and chicken parts

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Process risk models (PRM) predict consumer exposure and response to food produced by specific scenarios. A study was undertaken to develop a PRM for *Salmonella* and chicken parts that had four unit operations (pathogen events): 1) meal preparation (contamination); 2) cooking (death); 3) serving (cross-contamination); and 4) consumption (dose-response). The PRM was created in Excel and was simulated with @Risk. Enrichment real time PCR was used to acquire data for contamination of chicken parts with *Salmonella*. Prevalence of *Salmonella* on chicken parts (wings, breasts, thighs, drumsticks) at meal preparation was 16% (25/160) whereas incidence of cross-contamination of cooked chicken during serving was 12% (5/40). Six serotypes of *Salmonella* were isolated with most being Typhimurium var 5- (67%) and Typhimurium (20%). Number of *Salmonella* (minimum, mean, maximum) was 0, 0.45, 0.93 log on raw chicken parts and 0.11, 0.32, 0.68 log on cooked chicken. Pathogen events were simulated by linking a discrete distribution for incidence to a pert distribution for extent. A model that considered normal risk and high risk (HR) serotypes and consumers was used to predict dose-response. Sensitivity analysis indicated that salmonellosis occurred when cooked chicken was cross-contaminated with a HR serotype during serving and then consumed by a HR consumer. A scenario was simulated for the acquired data with incidence of undercooking, cross-contamination, and HR consumers set at 20%. One-hundred simulations with different random number generator seeds were done to assess uncertainty of PRM predictions. Simulation results indicated that cases of salmonellosis per 100,000 chicken parts fitted a normal distribution and ranged from 7 to 25 with a mean of 16. The PRM can be used to compare safety of different lots of chicken as well as identify factors that can be targets of interventions that reduce this risk to public health.

Keywords: process risk model, Salmonella, chicken parts

[O11.01]

A risk modelling approach for setting process hygiene criteria for Salmonella in pork cutting plants, based on enterococci

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Pork is known to be a key source of foodborne salmonellosis. Processing steps from slaughter to cutting and retail contribute to the *Salmonella* consumer exposure. In two extensive surveys comprising a total of 5,310 pork samples, cuttings and minced meat were analysed semi-quantitatively for *Salmonella* and quantitatively for the hygiene indicator enterococci. The samples were collected in 2001/2002 and 2010/2011 in Danish cutting plants, retail supermarkets and butcher shops. A positive correlation between prevalence of *Salmonella* and number of enterococci was shown (Hansen et al., 2013). As enterococci and *Salmonella* share a lower growth limit around 5°C, the positive correlation could imply that the meat had been exposed to temperatures above 5°C. Based on these findings, the objective of this study was to develop an approach for setting process hygiene criteria for predicting *Salmonella* risk in cutting plants from enterococci counts. The novel approach uses risk modelling to associate a relative consumer risk to different levels of enterococci in pork. The applied risk model was a modification of a model developed by Duarte et al. (submitted). The output is an estimate of the relative risk of acquiring salmonellosis associated to a given concentration of *Salmonella*. The relative risk of acquiring salmonellosis was then associated to the concentration of enterococci by using the observed positive correlation between *Salmonella* and enterococci as model input. From the applied model it was deduced how much the consumer risk can be reduced if enterococci is kept below a certain limit.

Hansen, T.B, G. Sandø and S. Aabo (2013). Correlation between *Salmonella* and hygiene indicators in the Danish fresh pork chain. In: Proceedings from Cold Chain-Management, 5th International Workshop, Bonn, 10-11 June 2013, paper 5.3.

Duarte, A.S.R., M.J. Nauta and S. Aabo (submitted). Variation in the effect of carcass decontamination impacts the risk for consumers.

Keywords: Proces hygiene criteria, Risk modelling, Salmonella, Pork meat

[O11.02]

Quantitative risk assessment of human infections with pathogenic E. coli from irrigation water to lettuce and additional risk posed by presence of extended spectrum and AmpC β-lactamases

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The contribution of the fresh produce production environment in human disease and mortality burden from transmission of pathogenic bacteria bearing extended spectrum β-lactamases and AmpC β-lactamases (ESBL/AmpC) has not been studied. We recently reported a high prevalence of ESBLs/AmpC as well as a high gene transfer efficiency of lettuce and irrigation water *E. coli* isolates with water isolates showing a significantly higher conjugation frequency than those from lettuce. This study aimed at undertaking a quantitative microbial risk analysis (QMRA) of infection, illness and mortality (IIM) in various consumer groups by ESBL/AmpC positive pathogenic *E. coli* contamination from irrigation water to lettuce.

The QMRA was constructed in Ms Excel spreadsheet with farm to consumption chain accounted for by modules including initial contamination, cross-contamination or dilution through mixing, growth or decline during transport, retail and domestic handling. Frequency of ESBL/AmpC transfer was determined using lettuce model studies. Portions of lettuce consumed were also taken into account. The dose-response was modeled using Beta-Poisson model to calculate the probability of IIM from pathogenic *E. coli*. Monte Carlo simulation was carried out using @Risk software. Risk was further divided into impact of pathogenic *E. coli* alone and effect of pathogenic *E. coli* given that they are ESBL/AmpC positive. Different consumer groups were taken into account due to differences in immunity and portions consumed. Sensitivity analysis was carried out to suggest most effective mitigation strategies of the pathogen events from farm to fork either singly or in combination. Important model assumptions were outlined. Considering disability-adjusted life year (DALYs) surveillance, diagnostics and laboratory costs, and the associated psycho-social effect of human death, preventing illnesses and deaths associated with ESBL/AmpC positive pathogenic *E. coli* will become imperative. Example public versus private partnerships were recommended. Efforts will be necessary for an effective antimicrobial resistance surveillance and treatment programme.

Keywords: Quantitative microbial risk assessment, Pathogenic E. coli, Lettuce, Extended spectrum and AmpC beta-lactamases

[O11.03]

The role of economics in food safety risk analysis

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Introduction: Food safety risk assessments provide a valuable tool for risk managers. Nevertheless, the information provided by such analyses is often presented in a manner that is not useful to policy makers. For example, alternative means of addressing pathogens in the environment may lead to differential, and often incomparable health effects across a population. The integration of economic evaluations into the risk analysis framework (ideally though direct integration into risk assessments) can make these comparisons possible, giving risk managers a powerful tool that can be used to help make cost-effective decisions.

Methods: This study demonstrates how economics fits into the risk analysis process as a component of risk assessment, risk communication, and risk management. A model for the economic cost of illness for foodborne illnesses is presented and examples of uses of economics in risk analysis are demonstrated. A Monte Carlo simulation model is used to illustrate the potential value of using economic analysis to assess interventions with alternative strategies for mitigating health losses associated with foodborne illness.

Results: Simulation results demonstrate that economic valuation of illness provides a way of normalizing benefits from risk reduction across multiple contexts and pathogens. Furthermore, the results show how integration of economic analysis into a risk assessment yields more precise benefits estimates.

Discussion: Economic analysis can be an important part of food safety risk analysis process. It is particularly useful as a tool to help risk managers make optimal science-based policy decisions. The novel result of this study is the generation of simulation results illustrating how integration of economic analysis into food safety risk assessment models can yield more precise benefits estimates than models that simply use the outputs of these models.

Keywords: Economics, Food Safety, Risk Assessment

[O11.04]

A web-based application customized to food safety requirements of small-sized enterprises

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Today, European legislation considers predictive microbiology as a tool to manage food safety. People in the food industry, including those in small-sized enterprises, even if not availing themselves of specific knowledge, are encouraged to use the same approach. To extend a bridge between both sides, a user-friendly simplified web-based software (*Praedicere Possumus*, PP) has been developed. Through this software, users have access to different modules. The first module provides the opportunity to evaluate the possibility of a number of pathogenic bacteria to growth, using a growth/no growth boundary model (Polese et al., 2011). The main environmental factors taken into account are: temperature, pH, a_w, organic acids, food additives and microbial interaction. The fractional contribution (f_c) of each inhibitory factor to growth probability (P) is estimated as a function of the difference between the actual level of the factor and the inhibiting value, adjusted for the sub-optimal interval of the factor. This option can assist users in defining processing and storage conditions to attain a desirable food safety level. If P>0.1 pathogen population density can be estimated with a kinetic module, which uses a three-phase linear model. The module also estimates thermal and non-thermal inactivation of the pathogens, and the parameter values are automatically reported. Quantification of the pathogen reduction helps users to identify the critical control points in a process. The thirth module, which takes into account the storage time in a time integrated probability parameter, allows user to evaluate the probability of growth related to a specific time. This module is expected to assist users in the determination of the shelf-life of food under the safety constraints. A number of models applied in PP are already validated and considered reliable for determining the compliance of a RTE product with EU safety criteria (Polese et al., 2014).

References

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Polese P., Del Torre M., Venir E., Stecchini M.L. 2014. Food Control 36, 166-173.

Keywords: Predictive microbiology, Food safety, Software, Food Business Operators

[O12.01]

Quantifying the impact of biological and experimental variability near the growth boundaries on the stochastic responses of growth, gene transcription and acid resistance of Listeria monocytogenes

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We aimed to evaluate the impact of experimental (E), biological (B) and technical variability (T) during culture preparation of *Listeria monocytogenes* on the stochastic outcome of: (i) growth (ii) acid resistance and (iii) relative transcription of stress- and virulence- associated genes, such as glutamate decarboxylase system (*gad2*) sigma factor B (*sigB*) and positive regulatory factor A (*prfA*), in response to pH and NaCl combinations near and across the growth/no growth interface of the organism at 7°C.

L. monocytogenes strain C_5 inocula were prepared from: i) different colonies (B), ii) same colony and different second activation (B) and iii) same colony and same second activation (T). In addition, experimental replicates (E) corresponded to independent reproductions (n=3) of the entire experimental set up. Tryptic Soy Broth supplemented with 0.6% w/v yeast extract (TSBYE) and various combinations of NaCl (0-8 % w/v) and pH (4.8-7.2) (HCl) was inoculated with ca. 10^7 CFU/mL of *L. monocytogenes* C_5 (serotype 4b) and stored at 7^oC. Growth was monitored via optical density (620 nm) in 96-well microplates for a 20-day period (n=15x3) and OD data were used (DMFit) to estimate μ_{max}. Quantitative Real time PCR was used to evaluate gene transcriptional changes (24h post inoculation) (n=5x3), while survival under subsequent acidic conditions (TSBYE pH 2.0, HCl, 37^oC, up to 35 min) was assessed using *D*-values determined by fitting the biphasic model without shoulder of GinaFiT freeware.

Experimental and biological replicates exhibited differences in mean estimates and variance of μ_{max}, $D_{pH:2.0}$-values and relative gene expression. Growth-limiting combinations of pH (5.0-5.2) and NaCl (2%) resulted in high experimental growth variability as expressed by the coefficient of variation (CV) for μ_{max} in the range of 15 to 60%, while the habituation of the bacterium in these conditions led to the highest $D1_{pH:2.0}$-values (2-10 min), suggesting acid adaptation, even though again with high variability (CV 15-40%). On the contrary, habituation of *L. monocytogenes* at mild pH (6.0-7.2) and elevated NaCl (4-6% w/v) resulted in lower maximum experimental and biological variability for μ_{max} ($CV\mu_{max} \leq 20\%$) and $D_{pH:2.}$ ($CV_{DpH:2.0} \leq 30\%$) as well as lack of acid adaptation, manifested by reduced resistance to lethal pH.

These findings could contribute to the stochastic assessment of *L. monocytogenes* survival and growth responses close to the growth boundaries and pinpoint the underlying concerns in experimental approaches assessing the stress response of pathogens under growth limiting conditions.

Keywords: Listeria monocytogenes, variability, stress response

[O12.02]

Effect of temperature abuse on freeze-thaw characteristics and microbial quality of frozen army rations: A numerical study

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Frozen army rations for the deployed US army units are manufactured in the US main land and shipped to army bases in various countries. During storage and transportation, these food items might get exposed to severe temperature fluctuations, which might lead to microbial spoilage. Numerical simulation, using COMSOL® Multiphysics software, was carried out to predict the effect of external temperature conditions on the thermal behavior and the microbial quality of selected frozen US army rations. An army breakfast menu box containing beefsteaks, orange juice, peppers & onions, French toasts, and Danishes, was selected for conducting this research. Thermo-physical properties of each food item were characterized using their composition and a differential scanning calorimeter (DSC). In the mathematical model, apparent specific heat method was used to account for the latent heat of phase change during thawing and refreezing. Numerically predicted thermal behavior results were experimentally validated using a gel-based model food system and the food items in the menu box.

Beefsteak was the most susceptible food in the menu box for microbial spoilage. A microbial predictive tool, ComBase database, was used to identify and evaluate the kinetics of the most prone microorganism that can grow in a beefsteak. The spoilage microorganism *Pseudomonas* spp. was considered to represent the fastest growing microorganism and its kinetics was combined with the numerical model to predict the microbial quality of army rations during two possible temperature abuse scenarios. Numerical predictions suggested that the food items exposed to external temperatures ranging from 20 °C to 40 °C, can be allowed to stay at those temperatures for a maximum times of 28 h to 11 h, respectively, and in the case of freezer failure, the food items can be allowed to stay inside the broken freezer for a maximum time of 186 h, to ensure microbial safety.

Keywords: numerical simulation, frozen food, temperature abuse, microbial spoilage prediction

[O12.03]

Inactivation kinetics of bacterial spoilers of beers during acid washing process of pitching yeast

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Acid washing of pitching yeast is used to reduce the load of spoilage bacteria and allow its reutilization. A better management of this process can be attained if inactivation kinetics of common beer spoilers is known. This may allow industries to select conditions for inactivation of contaminants of while keeping the viability of yeasts.

The main objective of this study was to determine the inactivation parameters of beer spoilage bacteria during simulated acid washing of pitching yeast.

Acid washing was simulated using phosphoric acid at two pH values (1.5 and 2.0). At the end of 12° Plato Lager fermentation, the yeast was cropped and re-suspended in a ratio of 1g yeast/5 mL beer. This mixture was inoculated with each of *Lactobacillus brevis* (DSM 6235) and *Lactobacillus casei* (ATCC 334) at 10^8 CFU/mL. Inoculated pitching yeast were maintained at 4°C and at different time intervals samples were collected for enumeration of yeasts (YPD, 25°C/3 days) and bacteria (MRS agar, 30°C/2-5 days). The number of survivors was then plotted against time and Ginafit was used to fit the data and estimate the inactivation kinetic parameters.

The mathematical model based on Weibull distribution accurately described the inactivation kinetics of both microorganisms. *L. brevis* and *L. casei* washed at pH 2.0 presented, respectively, a δ of 123.99 min and a *p* of 1.95 (R^2 0.99) and a δ of 5.36 min and a *p* of 0.83 (R^2 0.96). However, for the treatment at pH 1.5 the δ was 15.14 min and the *p* of 0.82 (R^2 0.93) for *L. brevis* and a δ of 0.91 min and a *p* of 0.49 (R^2 0.94) for *L. casei*. Since that, all treatments didn't change the viability of yeast, this finding and model presents new information to different approaches and strategies for acid washing pitching yeast.

Keywords: brewing, organic acid, spoilage, lactic acid bacteria

[O12.04]

Is food safety compatible with food waste prevention and sustainability of the food chain?

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In a context where the sustainability of food chains and food waste prevention are subjects of interest for public authorities and professionals, it is important to assess if these new objectives of food policy are compatible with food safety. All actors in the food chain (from food production to final consumer) have a role to play in these three aspects. The objective of this work is to develop a global model for a ready-to-eat meat product that provides three different outputs, i.e. energy consumption, percentage of spoiled products and exposure levels of *Listeria monocytogenes*.

First a cold chain model was developed. It allows generating time-temperature profiles as a function of (variable) operating conditions (e.g. ambient temperature, thermostat setting, airflow rate in display cabinet, thermal insulation of domestic refrigerator…). The cold chain model was then coupled with (i) predictive microbiology models for *L. monocytogenes* and spoilage bacteria and (ii) energy consumption simplified models for the cold equipment involved (display cabinet, refrigerated trucks, etc.).

Various scenarios were applied in order to explore the consequences of potential changes in consumers practices (e.g. better thermostat setting) or in cold chain equipment (e.g. in retail premises). The model helps to assess quantitatively the consequences of these changes on safety, food waste and energy cost. For example if the temperature in the domestic refrigerator was decreased by 2°C, consumers' exposure could be divided by two but it would increase energy consumption by 10%.

This global and quantitative approach could help policy makers in decision making.

Keywords: food waste, Energy cost