Predictive Modelling in Food

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Edited by
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PREFACE

During recent decades, food microbiologists have been investigating the diverse roles of microorganisms in different commodities. This gained knowledge has helped to better understand the key elements and underlying mechanisms behind microorganisms' behaviour in different situations. In this commitment, predictive microbiology has identified and shed light on gaps in broad-ranging areas of progress in the assurance of food quality and safety through the development of fit-for-purpose mathematical models. Since foods are essentially multi-element matrices where chemical, physical and microbiological components interact with each other, underlying factors affecting microbial growth and survival may still need to be elucidated.

The International Committee on Predictive Modelling in Food (www.icpmf.org), which was founded in 2011, has the mission to promote the development of predictive models and to generate new knowledge in the field that are relevant to food stakeholders, risk assessors and governmental authorities. This objective is primarily achieved through advancing the success and sustainability of the biennial ICPMF-conferences. The last ICPMF10 conference, held in Córdoba (26-29th September 2017), provided outstanding research studies which have contributed with important achievements in *the predictive modelling* field.

This book presents a number of selected communications presented at the 10th International Conference on Predictive Modelling in Foods, that are summarised into ten chapters, where the most relevant topics related to our area were addressed:

- Systems biology and whole-cell modelling.
- Individual-based models.
- Modelling approaches using (food) metagenomics data.
- Complex systems modelling approaches for food safety and quality.
- Modelling microbial dynamics in relation to food microstructure.
- Databases, software and decision-support tools in predictive modelling in foods.

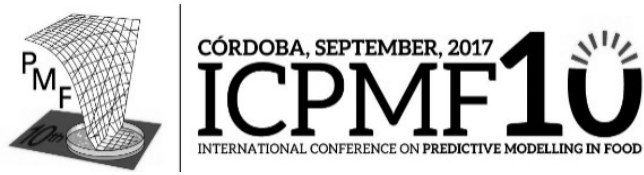
- Predictive models for food safety and quality: decontamination, food formulation, bacterial transfer, microbial spoilage, etc.
- Predictive models for food process simulation: dehydrating, mixing, forming, heat transfer, etc.
- Modelling the impact of microbiological interactions in foods.
- Interdisciplinary approaches and new advances in predictive modelling in foods.
- Quantitative Microbial Risk Assessment and Management.
- Predictive mycology.

We are convinced that *predictive modelling* is going a long way towards providing more and more accurate and useful estimations for food quality and safety assurance, since several scientific disciplines involved in *predictive modelling* are enjoying great development in the last years.

Predictive modelling in food will presumably become an immensely useful literature resource for graduate students, researchers, food authorities, risk assessors, managers, and stakeholders in food science, food microbiology, statistics, food engineering, and biotechnology.

We would like to thank all authors of the communications presented in this book for their invaluable scientific contribution in this field. We would also like to thank Mrs. Helen Cryer for giving us the opportunity to disseminate this stimulating research as well as the staff from Cambridge Scholars Publishing for offering excellent support throughout the editing process. All in all, we hope that the readers will enjoy the book and rather consider it as a useful tool, and why not, a source of inspiration.

THE ORGANISING COMMITTEE OF THE ICPMF10



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CHAPTER 1

INTERDISCIPLINARY APPROACHES AND NEW ADVANCES

MICROBIAL MODELLING COUPLING THE DYNAMICS OF DIFFUSED GASES AND MICROBIAL GROWTH IN MODIFIED ATMOSPHERE PACKAGING

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INTRODUCTION AND OBJECTIVES

Modified atmospheres (MA) packaging is widely used, to delay spoilage and extend the shelf-life of fresh products such as poultry and fish fillets. Predicting microbial safety of fresh products in modified atmosphere packaging implies taking into account the dynamics of O_2, CO_2 and N_2 exchanges in the system and its effect on microbial growth. The bacteriostatic effect of CO_2 within MAP is primarily influenced by CO_2 absorption into the food. The same stands for O_2, especially when dealing with microaerophilic microorganisms. Therefore, microbial dynamics should be built considering the concentration of the dissolved gases.

In this work, a microbial model describing the dynamics of *Pseudomonas* spp. coupling diffused gas concentrations of CO_2 and O_2 is developed.

MATERIAL AND METHODS

Previously published data of skinless chicken fillets stored in gaseous mixtures of 10%, 30%, 50%, 70% and 90% CO_2 balanced with N_2, 80:20% O_2:N_2 and 40:30:30% CO_2:O_2:N_2 and control conditions (air) at 2 °C were used (Bolton *et al*., 2014). The carbon dioxide solubility was obtained by monitoring the changes in the headspace volume over time using a buoyancy technique and performing calculations based on volumetric measurements and the Henry's constant. Henry's constant was also used to estimate the oxygen solubility in the chicken fillets. The microbial model

was built by extending the elementary model building block of the two first order differential equations for the Baranyi model (Baranyi and Roberts, 1994). The dependence of the maximum specific growth rate on the microbial adaptation, and the diffused CO_2 and O_2 concentrations were introduced.

A total of seven parameters were in the combined primary and secondary models: $\beta1 = \mu_{opt}$ (1/day), optimal growth rate; $\beta2 = CO_{2\ max\text{-}diss}$ (ppm), maximum concentration of $CO_{2\ dissolved}$ (ppm); $\beta3 = O_{2\ ref}$, dissolved O_2 in water at 760 mm HG, $\beta4 = O_{2\ min}$, minimum O_2 concentration (ppm); $\beta5 = N_{max}$, maximum microbial concentration (CFU/mL); $\beta6 = N_0$, initial microbial concentration (CFU/mL); $\beta7 = Q(0)$ (CFU/mL), parameter having to do with the microbial physiology.

RESULTS

A plot of scaled sensitivity coefficients for the parameters revealed that only two parameters could be estimated. For each combination of gas mixtures, μ_{opt} and $CO_{2\ max\text{-}diss}$ were estimated from the dynamic data. The values of μ_{opt} ranged from 1.5 to 10.7 (1/day), increasing exponentially with N_2 concentration. The values of $CO_{2\ max\text{-}diss}$ ranged from 255 to 3100 ppm, decreasing linearly with N_2 concentration. The relative errors of the parameter estimates ranged from 0.38 to 10.5%. The RMSE of the fits of logN to time ranged from 0.27 to 0.71 log (CFU/mL) out of a 4-9 log scale.

CONCLUSIONS

These favourable parameter estimation results hold promise for the development of more comprehensive models for global regression for relevant dynamic data.

ZERO-INFLATED REGRESSIONS FOR MODELLING MICROBIAL LOW PREVALENCE AND SAMPLING PERFORMANCE FOR FOODBORNE PATHOGENS

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INTRODUCTION AND OBJECTIVES

Microbial contamination of raw poultry meat could occur because of improper handling at primary production and slaughterhouse levels. Low microbial prevalence data often consist of a high amount of non-detections (zero positives), so a flexible framework is required to characterise the underlying microbial distribution and conduct reliable inferential statistics. Thus, the objective of this work was to evaluate the performance of Zero-Inflated Binomial (ZIB) regression models to describe the effects of sampling site (carcass, thigh, breast, wings) on the measured incidences of *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus* on chicken meat. For this aim, a number of fixed- and random effects models were evaluated and compared, while sampling performance based on mean prevalence estimates was assessed.

MATERIAL AND METHODS

Poultry samples were taken during three consecutive years from a Spanish slaughterhouse (36 sampled batches from 144 sampling periods). Analyses were carried following ISO methods for each pathogen taking 25 g samples from each site. Carcass samples were collected before jointing, while thigh, breast and wings were sampled afterwards. For each pathogen, four ZIB models were fitted to the presence/absence data with sampling site as covariate and random-effects due to sampling occasion either in the binomial probability (p) or in the extra proportion of zero counts (w0). Models were fitted using the Markov chain Monte Carlo (MCMC) technique via WINBUGS 1.4.3.

RESULTS

The data sets of the three pathogens presented a high proportion of non-detections. While *Salmonella* spp. was the pathogen least frequently detected from poultry meat (90.9% non-detections), *L. monocytogenes* and *S. aureus* gave positive results slightly more often (14-15% detections); although in general the frequency of positive results was conditional upon the sampling site. For the three data sets, the sampling site exerted a greater effect on the extra proportion of non-detections than on the binomial prevalence itself, with breast bearing the lowest prevalence estimates of *Salmonella* spp. (mean: 0.0088; 95% CI: 0.0002-0.0195) and *S. aureus* (mean 0.0148; 95% CI: 0.0001-0.0400). The fitting capacity of the models was further improved when random effects due to sampling occasion were placed in the extra proportion of non-detections (deviances decreased from 146.7-156.7 to 140.2-140.6). At any sampling site (breast, carcass, thigh or wings), the mean prevalence was estimated as 0.0135 (95% CI: 0.0015–0.0270) for *Salmonella*, 0.0211 (95% CI: 0.0004 – 0.0563) for *L. monocytogenes* and 0.0236 (95% CI: 0.0004 – 0.0512) for *S. aureus*.

CONCLUSIONS

Under a ZIB assumption, most of the variability in the occurrence of pathogens on chicken meat was found to lie in the process producing the extra proportion of zero counts rather than in the binomial probability itself. With the basis on an adequate description of microbial contamination, sampling procedures of poultry meat can be effectively addressed.

PERFORMANCE OF MICROBIOLOGICAL CRITERIA FOR HYGIENE CONTROL OF *CAMPYLOBACTER* IN GERMAN SLAUGHTERHOUSES

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INTRODUCTION AND OBJECTIVES

Campylobacter is mostly considered as an important foodborne pathogen having a high impact on the public health burden. Source allocation studies identified broiler meat as the most important single food transmission vehicle of *Campylobacter*. In this regard, microbiological targets are under development in the EU and characterisation of microbial contamination seems to be an essential part to implement sampling schemes.

The aim of this study was to analyse broiler batches processed at three conventional slaughterhouses in Germany for their *Campylobacter* load at the end of processing. Microbial contamination of studied flocks was assessed through the comparison of Log normal, Poisson-log normal and Negative binomial distributions. Sampling procedures were subsequently applied to assess batch acceptability.

MATERIAL AND METHODS

Samples of broiler neck skin and caecal content (356 batches) were collected in three German slaughterhouses (A, B and C) for fresh meat production from conventional reared broilers over a period of 36 months from July 2013 to June 2016. Microbial analyses of five-pooled neck skin samples and one-pool of caeca samples out of ten individual samples were performed in accordance with ISO 10272-2. Different concentration distributions (i.e. Log normal, Poisson-log normal and Negative binomial) were fitted to observed data through MLE by using R v3.2.3 (*fitdistrplus* for censored data and *poilog* packages). Sampling plan performance was assessed by setting 500 and 1000 CFU/g as microbial limits.

RESULTS

In this study, the individual prevalence of *Campylobacter* positive broiler batches processed at the three slaughterhouses ranged between 21.7% (95% CL: 21.1–22.3%) and 47.9% (95% CL: 47.07–48.71%). Significant differences among mean *Campylobacter* counts in neck skin samples were denoted according to the slaughterhouse evaluated ($P \leq 0.05$). The results showed that distributions were left-shifted thus indicating a high proportion of low microbial counts in the samples. Negative binomial regression provided better adjustment at low contamination levels.

CONCLUSIONS

The results obtained will help food business operators to evaluate the status of microbiological hygiene and safety in relation to *Campylobacter* contamination, and also will assist them in setting their own acceptable microbial limits for process improvement with consideration to existing legal requirements.

GROWTH MODELLING OF *WEISSELLA VIRIDESCENS* BY REAL-TIME QUANTITATIVE PCR (QPCR)

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INTRODUCTION AND OBJECTIVES

The lactic acid bacteria (LAB) *Weissella viridescens* has been reported as responsible for spoilage of meat products. To identify, quantify and model this bacterium growth in culture medium, a SYBR-Green Real-Time Quantitative PCR (qPCR) procedure has been developed, using the *recN* gene as a target.

MATERIAL AND METHODS

To confirm the efficiency and sensitivity of the SYBR-Green based assay, a melting curve analysis was performed to check the specificity of the amplification reaction during the qPCR analysis. The growth curve for a *W. viridescens* ATCC 12706^T pure culture enumerated by qPCR was compared with the one enumerated by plate counts. The experiment was conducted in optimal growth temperature (30 °C) until the stationary phase. Baranyi and Roberts' model was fitted to growth data using Matlab® software.

RESULTS

The results demonstrated that the primers were specific for *W. viridescens* with specific signals in melting temperatures of 81.3 ± 0.1 °C for this species. Standard curves presented efficiency values of 112% and suitable correlation coefficients ($R^2 > 0.99$). The limit of detection was found to be 1.2 log DNA copy number that corresponds to 3.7 log CFU, a suitable CFU enumeration range for spoilage LAB, considering that they should be initially present in meat products. The statistically significant difference (p-value of < 0.05) between qPCR and plate count was observed only during the exponential phase (15 hours of cultivation), corresponding to 7.95 and 7.6 log CFU, respectively. The model presented a good fit ($R^2 > 0.99$) for