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Paris 2013
September 16-20
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Predictive microbiology in food: Today's tools to meet stakeholders' expectations

Proceedings

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8th International Conference on Predictive Modelling in Food

ICPMF8, September 16-20, 2013, Paris, France

Institute Pasteur, 28 rue du docteur Roux, 75015, Paris

CONFERENCE PROCEEDINGS

Predictive microbiology in food:
Today's tools to meet stakeholders' expectations

www.icpmf8.org

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CNIEL, IFIP

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WELCOME

Dear Colleagues,

As representatives of IFIP - The Institute for pig and pork products - , the Sym'Previus scientific interest group and the CNIEL - the French dairy board - , we are pleased to welcome you to the 8th International Conference on Predictive Modeling in Food, Paris, September, 16-20, 2013.

Our aim is to bring together food processors, innovative researchers and young scientists working to enhance food quality and safety with a special focus on the transfer activities towards food business operators. We propose to address the global challenges that predictive modelers and risk assessors are facing in the food quality and safety field.

With its rich program, we hope that the conference will allow fructifying scientific exchanges, networking and transfer activities towards food business operators with:

- four main topics through 10 plenary oral presentation sessions and 4 poster sessions,
- a special day dedicated to food business operators with a software fair,
- a ½ day workshop to discover innovative methods in predictive microbiology,
- a full day workshop dedicated to risk-based control measures in food establishments
- a ½ day presentation of French and international research projects,
- a technical tour to Rungis, the largest fresh produce market in in the world

We express our gratitude to our sponsors for their support and thank you for your valuable contribution.

We hope you will enjoy the meeting and side events and have a pleasant stay in Paris!

The Chairs of the 8th International Conference on Predictive Modelling in Food.

Mariem ELLOUZE, Frédéric CARLIN and Fanny TENENHAUS-AZIZA.

SCIENTIFIC AND NATIONAL ORGANIZING COMMITEES

This conference is organized by:

The French institute for pig and pork products (IFIP)

The Sym'Previus scientific interest group

The French dairy board (CNIEL)

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CONFERENCE SNAPSHOT

	Monday, Sept. 16th 2013	Tuesday, Sept. 17th 2013	Wednesday, Sept. 18th 2013	Thursday, Sept. 19th 2013	Friday, Sept. 20th 2013
Morning sessions	*Workshop 2 registration*	*ICPMF8 registration*	*Software Fair registration*		*Technical tour to Rungis (starting at 4am)*
	Workshop 2	Session 1: Data collection (1/2)	Session 5: Application of predictive microbiology for food industry and risk managers (1/2)	Session 7: Advances in methods & models in predictive microbiology (1/3)	Research projects Seminar: Session 1
	Coffee break	*Coffee break / Poster session*	*Coffee break / Poster session*	*Coffee break / Poster session*	*Coffee break*
	Workshop 2	Session 2: Data collection (2/2)	Session 6: Application of predictive microbiology for food industry and risk managers (2/2)	Session 8: Advances in methods & models in predictive microbiology (2/3)	Research projects Seminar: Session 2
	Workshop 1 registration	*Lunch*	*Lunch*	*Lunch*	*Lunch*
Afternoon sessions	Workshop 1	Workshop 2	Session 3: Predictive models for food quality and safety (1/3)	Software Fair : Presentation session	Session 9: Advances in methods & models in predictive microbiology (3/3)
	Coffee break	*Coffee break*	*Coffee break / Poster session*	*Coffee break*	*Coffee break*
	Workshop 1	Workshop 2	Session 4: Predictive models for food quality and safety (2/3)	Software Fair : Demonstration session	Session 10: Predictive models for food quality and safety (3/3)
Evening events	*Welcoming coktail and ICPMF8 registration*		*Gala Dinner*		

*** for exact sheduling and location, please consult the detailed program**

LIST OF ABSTRACTS

DATA COLLECTION

Oral conferences

Bacterial Economics. Predictive modeling in food sciences should, and does, borrow ideas from fellow sciences

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OBJECTIVE

This talk first gives a short overview of the evolution of predictive modeling in food science in the last thirty years, drawing a parallel to similar developments in fellow-sciences. It will be pointed out that universality and specificity are central in this process. For demonstration, models for bacterial inactivation, survival, adaptation and growth will be discussed, and shown to have analogous features to those describing human activities. Such analogies help to understand the way mathematical models are created and can inspire new developments.

METHODS

Mathematical modeling is an “art of omitting the unnecessary”, after which the remaining backbone can be a common denominator for various problems in life sciences, while the *applications* of the models inevitably highlight the differences between those fields. This will be shown by several examples drawn from the field of modeling bacterial kinetics.

RESULTS

Bacterial cell is a "biotechnological factory", embedded in its environment, characterized by media/food, chemicals, interactions with other cells and species, just as how humans are embedded in their environment, economy and society. Analogies can be detected between the ways how bacterial and human economies respond to environmental changes. Not surprisingly, the respective mathematical models show similarities. However, when applying the models to experimental design, parameter identification/estimation or just simple simulation, pragmatic considerations (experimental feasibility, numerical and statistical optimization) can play significant role in their implementation.
Here we show examples for this, when modeling transition phases, when analyzing complex bacterial networks and when comparing survival and growth strategies.

CONCLUSIONS AND IMPACT OF THE STUDY

Mathematical models can link fellow scientific fields while their implementation highlights the differences between those fields. Recognition of such differences helps predictive microbiology to find its own niche in food science.

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Ercsey-Ravasz MM, Toroczkai Z, Lakner Z, Baranyi J (2012): Complexity of the International Agro-Food Trade Network and its Impact on Food Safety. PloS One 7(5) e37810

József Baranyi is the Research Leader of the Computational Microbiology Research Group, leads the ComBase National Capability of the Institute of Food Research, Norwich, UK. He is also a Visiting Professor at the Imperial College, London.

Dr Baranyi's research focuses on the mathematical modelling of microbial responses to food environments. He has been editorial board member of Applied and Environmental Microbiology since 1998, editor of BMC Microbial Informatics and Experimentation since 2010 and was the statistical advisor of the Journal of Applied Microbiology between 1996 and 2010.

Dr Baranyi has developed several software tools (notably pioneered the ComBase system, an internet-based combined database and predictive software tool), has written 70 peer-reviewed articles, with a total citation of 3000. His mathematical model on bacterial lag published 20 years ago is commonly referred to as the Baranyi-model in the literature.

Variability and uncertainty in the evaluation of predictive models with literature data

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OBJECTIVE

Predictive models of microbial growth are important tools for quantitative microbial risk assessment (QMRA) (Nauta, 2000). However, for reliable predictions, models must be validated for the product of concern and realistic conditions of contamination and handling (Ross et al., 2000).

As a simplification of reality, models have a limited predictive ability (Ross et al., 2000), and the growth settings covered by a model represent one of the factors that may contribute to inaccurate predictions.

Many studies have shown that a change in experimental settings, such as the bacterial strain or the growth media, leads to different estimates of growth parameters or to different 'performance' of the same model. Consequently, stochastic integration of variability of microbial growth is indispensable for the application of predictive models in QMRA. Variability can be defined when model validation is performed under well-controlled conditions, such as challenge studies: sources of variability are more difficult to define when a model is validated against literature data. In this case, to characterize variability or uncertainty associated to predictions, it is crucial to determine the effect of data-related factors on model performance.

Here we assess how different growth settings inherent to literature datasets affect the performance of a growth model compared to its performance with the data used to generate it.

METHODS

We use the accuracy factor (AF) Ross (1996) as a measure of model performance, a published square root-type model for the growth rate of *Escherichia* coli in response to four environmental factors (Ross et al., 2003) and literature data that have been previously used to evaluate it (Mellefont et al., 2003). When assessed against the data used to generate it, the model has an AF of 1.21, which is here assumed as its best possible performance.

For each evaluation dataset, we registered the number of observations and the ranges of temperature (T), water activity (a_w) and pH under which they were made. We also registered the presence or absence of lactic acid, the use of a pathogenic or non-pathogenic strain and the growth environment.

We compare graphically and statistically the distribution of AF values of data generated under different experimental settings, by testing the hypothesis that mean and variance is lower in groups for which conditions are closer to those of the original data used to generate the model. We assess the difference in variances using a one-sided F-test, and in the cases where no statistical significance is found, the difference in means with ANOVA.

RESULTS

The results show that selecting large datasets and datasets produced with a growth environment comparable to that used to generate the original data has more impact on the reduction of performance variation than

selecting datasets with a similar bacterial strain or the complete overlap between the growth conditions and the interpolation region of the model.

CONCLUSIONS AND IMPACT OF THE STUDY

This study supports the need for criteria for the choice of appropriate literature data to be used for model evaluation, in order to achieve a transparent and reliable model evaluation process.

ACKNOWLEDGEMENTS

This work resulted of a research stay at the University of Tasmania of author A.S.R. Duarte, which was possible due to a grant from the Norma and Frode S. Jacobsens Fond.

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SORF: An Interactive Web-Based Application for Facilitating Data Management, Exchange and Analysis in Predictive Modelling Research

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OBJECTIVE(S)

Web-based applications and online data repositories have played a major role in recent years in advancing food-related research, by providing scientists and researchers with means of knowledge sharing, communication and data exchange, despite of their dispersed geographical locations. The Symbiosis Online Research Framework (SORF) was developed to provide the food research community with an interactive web application for data management, exchange, visualisation and analysis.

METHODS(S)

SORF is a web-based application that supports a wide variety of experimental platforms thanks to its generic entity-attribute-value (EAV) database backend [1]. The generic nature of the EAV allows the framework to manage heterogeneous data types without the need to provide separate storage modules for every experimental platform applied. The SORF makes data management straightforward despite the complex relationships typically found in food research conducted on a systems-level, where information is collected on a multiple knowledge level. Each experiment within the SORF framework is a multi-dimentional entity that can include one or more datasets as well as the experiment metadata. In addition to data management facilities, SORF also provides means for data visualisation and exploratory data analysis, by deploying the latest interactive Web-based technologies such Ajax and JSF2.1 [2].

RESULTS

The recently developed SORF front end uses the latest web technologies to allow users to upload, query, browse, and visualise data through a series of data views, exploratory data analysis tools and relational visualisations. This includes a series of interactive quality assessment plots generated on-the-fly using the data uploaded by the user, as well as multivariate analysis tools just as principal component analysis and 2D hierarchical clustering heatmaps. The interface provide access to a series of freshness profiles prediction models (193 Support Vector Machines in total) available online for users to predict the sensory score and bacterial counts for their uploaded analytical data (currently supporting HPLC and Electronic Nose data) [3]. The application is currently being used as a central data repository for the Symbiosis-EU project, which aims to provide novel methodologies for assessing freshness in meat products.

CONCLUSIONS AND IMPACT OF THE STUDY

Symbiosis-EU serves as a perfect case study for SORF application, as it involves the application of various metabolic and proteomic techniques, such as HPLC, FTIR, machine olfaction, microarrays and mass spectrometry. In addition, all acquired analytical data is accompanied by experimental metadata and results from traditional microbiological methods such as bacterial counts and sensory scores.

Due to the generic design of the SORF database backend, and its interactive user-friendly interface, the system can be easily adapted for other food-related projects, where means of data storage, exchange, and analysis are required.

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Semi-automatic loading of a microbial risk in food database thanks to an ontology in the context of Linked Data

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OBJECTIVE(S)

A preliminary step in microbial risk assessment in food is to gather and to capitalize experimental data in order to feed simulation models. We have designed, in the predictive modeling platform Sym'Previus (www.symprevius.org), a relational database, MicroRisk-RDB (*Leporq et al. 2005*), to store experimental data in microbial risk. Data capitalization task encounters a challenging lock. Indeed, original data are spread out in heterogeneous data sources (scientific papers, technical reports / sheets, PhD thesis …). Moreover, they are expressed in heterogeneous formats (mainly tables, text and graphics) and vocabularies. Manual entering of data in a database (e.g. MicroRisk-RDB) is therefore a time-consuming task. We present in this paper methods and tools to facilitate data capitalization and reusability in the framework of the Linked Data initiative[1], which promotes the publication and connection of structured data on the Web.

METHODS

We are currently designing @Web-Tool[2], a semi-automatic tool guided by an ontology, to help domain experts to populate a database (e.g. MicroRisk-RDB) with data tables found in scientific publications, especially on the Web. The notion of 'ontology' is one of the key-stone of the Linked Data initiative and more generally of the Semantic Web. It is a vocabulary used to organize the knowledge of a domain of interest, so that data can be exported, translated, queried, and unified across independently developed systems and services[3]. In @Web-Tool, the user first downloads an HTML scientific document, and then data tables are semi-automatically identified and extracted. A graphical user-friendly interface helps the user to annotate data tables thanks to the same ontology used to index the database to be populated. The user may manually suggest new candidate terms to be added in the ontology which are managed by the administrator. @Web-Tool has been designed in the framework of the Linked Data initiative using the W3C standards stack (*Berners-Lee et al. 2001*).

RESULTS

@Web-Tool is currently tested by different experts in the framework of the French ANR project MAP'OPT (Equilibrium Gas Composition in Modified Atmosphere Packaging and Food Quality) in order to collect data on food packaging permeabilities used to develop new growth simulation model taking into account modified atmosphere conditions. Our tests rely on the MAP'OPT-Onto ontology (*Touhami et al. 2011*),

[1] http://linkeddata.org/
[2] http://www.paris.inra.fr/metarisk/research_unit/knowledge_engineering/softwares/web__1
[3] http://tomgruber.org/writing/ontology-definition-2007.htm

which has been built as an extension of the ontology used to index MicroRisk-RDB. Current version of MAP'OPT-Onto is composed of 1103 concepts representing domain objects (food products, microorganisms, and packagings), 23 quantity concepts and 83 associated units of measure. Finally 20 relation concepts are modeling the interdependence between domain objects and quantities (e.g. Temperature_Cardinal_Value relation concept express the relation between a given microorganism and its temperature conditions of growth).

CONCLUSIONS AND IMPACT OF THE STUDY

@Web-Tool is designed as a microbial risk in food data semantic Web annotation tool allowing a better reusability of experimental data in the framework of the Linked Data initiative. Our aim is eventually to publish MAP'OPT-Onto on the Web in order to set up a standardized vocabulary in microbial risk in food. In the very next future, we will link our MAP'OPT-Onto with international standardized ontologies, such as AGROVOC[4], the FAO thesaurus for agriculture, OM (*Rijgersberg and al. 2011*) and QUDT (*Hodgson et al. 2011*), in order to promote international data sources interconnection. Linking our MAP'OPT-Onto with other ontologies can provide us a richer definition of concepts we manipulate, more experimental data, access to conversion tools of OM and, so, a refined way of annotating and reusing the knowledge gathered. New bridges will therefore be established between data that will benefit to the simulation models. Finally, future evolutions of @Web-Tool will enhance data manual annotation efficiency thanks to semi-automatic annotation algorithms of data tables (*Buche at al., 2013*).

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Influence of gel microstructures on growth parameters of Escherichia coli in gelatin-dextran systems

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OBJECTIVES

Predictive models based on experiments in liquid are often used to predict microbial behavior in structured food systems. Nevertheless, food structure is reported to influence the microbial behavior. Bacterial behavior in structured environments is studied in different gelled systems and for several microorganisms [7]. However, most studies performing growth experiments use a single gelling agent, resulting in a homogeneous growth environment. In contrast, food products are often heterogeneous and consist of different phases. Observation of bacterial growth in heterogeneous model systems is already performed on randomly packed beds of glass beads, micro-porous silica particles and Sephadex microspheres [4], oil-in-water [3,5] and water-in-oil emulsion systems [6]. It has been shown that these model systems influence the behavior of bacteria [3,5].

In [2], the authors studied the microbial distribution in a variety of heterogeneous microstructures with different combination of gelatin and dextran. Regardless the gel microstructure, the preferential phase for microbial proliferation was shown to be the dextran phase.

To correctly predict the behavior of bacteria in food products, the effect of the microstructure on microbial dynamics must be studied thoroughly. With this aim, static experiments are performed in brain heart infusion (BHI) structured with seven binary mixtures of gelatin and dextran which possess different heterogeneous microstructures. In order to assess the effect of the heterogeneous gel microstructure, experiments are also performed in five singular gel systems with homogeneous microstructures.

METHODS

Experiments are carried out with *Escherichia coli* JM-109 DE3 pRSETb-Venus. Media are obtained by mixing BHI with different ratios of gelatin and dextran. Samples are prepared by filling spectrophotometer tubes with 1mL of inoculated medium. The tubes are placed in a temperature controlled water bath at 23.5°C, and at regular times one tube of each mixture is removed. The gels are melted at 37°C and cell density is determined via plate counting. Growth curves are fitted with the Baranyi growth model [1].

RESULTS

For all systems, typical growth curves are obtained. No significant differences were found among the lag phase and the maximum growth rate estimated for the seven binary mixtures. However, the maximum cell density (N_{max}) achieved at the stationary growth phase is strongly dependent on the gel mixture. An increase in gelatin or dextran causes a decrease in N_{max}. As the preferential phase for growth is the dextran phase, it was expected that the lag phase and the growth rate in the (singular) dextran mixtures would be similar to the parameters observed in the binary systems. However, a significant difference in lag phase and maximum growth rate was observed, illustrating that the effect of the surrounding microstructure cannot be neglected. In contrast, lag phase and maximum growth rate of singular gelatinsystems and binary mixture are similar. However, for the singular gelatin system, a reduced N_{max} level is observed.

CONCLUSIONS AND IMPACT OF THE STUDY

The results illustrate the possible effect of the surrounding microstructure on microbial dynamics. Additionally, it is demonstrated that, although bacterial growth still occurs in the same phase as in a homogeneous system, the presence of a heterogeneous microstructure influences the microbial behavior. This knowledge must be taken into account when predicting the bacterial behavior in real food.

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Combining predictive microbiology with cold-enrichment in minced pork for quantifying low levels of Salmonella Typhimurium DT104

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OBJECTIVE

For determination of *Salmonella* concentration in meat various methods can be used depending on the expected level. When higher levels (10^2 to 10^3 bacteria or more per g) are anticipated, plate count techniques using selective agars, i.e. XLD, are appropriate whereas for low numbers (3 to 10^2 bacteria per g) a most probable number (MPN) method is recommended. Recently, a real-time PCR-based tool for determination of concentrations as low as 1.4 *Salmonella* per 20 cm (*approx*. 10 g) of cork-borer samples of pig carcasses has been developed (Krämer et al. 2011). However, compared to plate count techniques the MPN and real-time PCR methods are very labour intensive and might not be suitable when analysing many samples within a short timeframe. We suggest to use enrichment in the meat at relatively low temperatures (11 to 16°C) combined with predictive models as an alternative. Therefore, the objective of this study was to evaluate the possibility to carry out a *Salmonella* enrichment step in the meat itself and use the two species interaction model, presented by Møller et al. (2013), for quantifying levels of *Salmonella* Typhimurium DT104 in minced pork.

METHODS

A total number of 101 minced pork samples were inoculated artificially with various concentrations (from 10 to 10^6 bacteria per g) of stationary phase *S.* Typhimurium DT104. Counts of *S.* Typhimurium DT104 as well as natural microbiota in the samples were determined immediately after inoculation and again after an enrichment step in the minced meat for *approx.* 48 h at 11 – 16°C. A rearranged version of the expanded Jameson-effect species interaction model, suggested by Møller et al. (2013), was applied for prediction of the *Salmonella* concentration in the minced pork samples. Observed and predicted counts of *S.* Typhimurium DT104 ($\log_{10}$-units) prior to enrichment were compared visually and by the acceptable prediction zone (APZ) method, *i.e.* percentage of predictions being within ±0.5 $\log_{10}$-units of observed values.

RESULTS

A relatively good agreement between predicted and observed values was seen. However, only 56 % of the predictions were within the APZ. The model tended to over-predict counts from 3 log-units and above, whereas under-prediction to some extent was seen for counts below 3 $\log_{10}$-units. Over-prediction was most likely explained by uncertainty of the lag-time model for *Salmonella*, i.e. a short lag time would result in a lower initial count to get to the same count after enrichment as compared to a long lag time. In contrast, part of the under-prediction appeared to coincide with competitive growth of *Citrobacter braakii* and *Hafnia alvei* on the selective agar. Therefore, under-prediction more likely resulted from underestimation of the *Salmonella* count after enrichment. Whether competition between these species also took place in the meat during enrichment is not known.

However, as the observed levels of the competitive species were below 5.5 $\log_{10}$-units it is questionable whether interaction with *S.* Typhimurium DT104 in the pork could have occurred. Omitting these samples and using the 56 observations below 3 $\log_{10}$-units improved the percentage of predictions within APZ to 63 %.

CONCLUSIONS AND IMPACT OF THE STUDY

A novel approach for determining *Salmonella* counts at low concentrations was proposed. Applying a simple plate count method, after cold enrichment (11-16°C for 2 d) in the pork sample itself in combination with predictive growth models, showed promising results. It indicates the potential of this approach as an alternative to meet the need for more sensitive methods, which are simple enough to be used in large-scale series of analysis.

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Assessment of microbiological quality and authenticity of minced meat using multispectral image analysis

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OBJECTIVES

So far, more than 50 chemical, physical and microbiological methods have been proposed for the detection and measurement of meat spoilage. It should be noted however that most of these methods are time-consuming and provide retrospective information, and as such they cannot be used in the meat distribution chain (Nychas et al., 2008). This issue was found to be harder due to the use of advanced preservation technologies [e.g., vacuum packaging (VP), modified atmosphere packaging (MAP), active packaging, etc] as well as the misuse or even abuse of storage temperatures in the meat chain. The use of microbial metabolites as well as the quantification of fingerprinting, e.g. with vibrational spectroscopy instruments and multispectral image analysis have been applied for assessing the quantitative distribution of different tissues in the surface of hams prior to salting or the quality of pork (Dissing et al., 2013).In particular, multispectral images can provide not only spatial information, as in the case of regular imaging systems, but also spectral information for each pixel in an image. Thus, using hyperspectral images, it is possible to assess physical and geometric characteristics such as color, size, shape and texture.

METHODS

More than 300 samples (21 different batches of minced meat from pork, beef and mix; 21 x 5 replicates x 3 types of meat) were analysed microbiologically (e.g. total viable counts (TVC), Enterobacteriaceae, lactic acid bacteria, pseudomonads, and *Brochothrix thermosphacta* (Dissing et al. 2013). In parallel snapshots using VideometerLab, a multispectral instrument, were acquired. The data acquisition was done using VideometerLab, which acquires multi-spectral images in 18 different wavelengths ranging from 405 to 970 nm. The spectral radiation of the 18 bands is not uniformly distributed over the sampling area, but rather at wavelengths of 405, 435, 450, 470, 505, 525, 570, 590, 630, 645, 660, 700, 850, 870, 890, 910, 940 and 970 nm. The acquisition system records surface reflections with a standard monochrome charge coupled device chip, nested in a Point Grey Scorpion camera. In this study, Principal Component Regression with leave-one-out cross-validation was employed to associate the counts of the microbial groups with multispectral images in an attempt to assess the microbial population directly from the acquired multispectral images. Moreover, the authenticity of the three types of minced meat was assayed directly from the images using Linear Discriminant Analysis (LDA).

RESULTS

The microbial association of the initial microbiota of minced pork and beef consisted of *Pseudomonas* spp., *B. thermosphacta*, lactic acid bacteria and Enterobacteriaceae. The developed model was found to be more accurate for TVC prediction in the caseof meat mix, yielding an accuracy of 1.07 and a standard error of prediction (SEP) of 9.09% after data scaling, whereas the largest SEP (23.93%) was observed in the case of lactic acid bacteria in pork.

It should be noted that the standard error of prediction of TVC was 12.42% and 11.66% for minced beef and pork, respectively. Excellent classification was evident when images from minced pork and beef were analyzed, as only one out of 39 beef samples was classified as mix and all pork samples were classified

correctly. In the case of mix, six out of 35 samples were incorrectly classified as beef, yielding an overall correct classification of 93.58%.

CONCLUSIONS AND IMPACT OF THE STUDY

The results of this study demonstrate for the first time that multispectral images in tandem with multivariate data analysis can be used reliably and accurately for the rapid assessment of meat microbiological quality.

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Multiple regression model of thermal inactivation of Salmonella enterica serovars in fluids. Significant effects of water activitity, sugar and pH

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OBJECTIVE

The variability of thermal inactivation kinetics can be estimated in a meta-model from literature data (Van Asselt & Zwietering, 2006). Differences in heating matrices and processing conditions however are known to cause overestimation of the variability. The objective of this study is to create a multiple regression model to investigate the variability of thermal inactivation in liquid foods resulting from differences in conditions that are uncertain and not under our control (e.g. the *Salmonella* serovar in the raw material) while adjusting for the effects of variables of the food matrix and processing conditions that are known and can be controlled. Water activity (a_w) was hypothesised to have a significant effect.

METHODS

Results of heat inactivation experiments under varying conditions were collected from literature (in total 514 data sets). The database of Van Asselt & Zwietering (2006) was used as a starting point, supplemented with data from more recent studies into the effects of low water activity in fluids. Data on heating menstrua and processing conditions were supplemented from the original literature or from other literature and internet sources. Only data sets with information on aw were used. The method was described earlier by Van Lieverloo *et al*. (2013).

RESULTS

All data together showed very large variability (R^2 = 0.2%, s.e. = 0.906). The effect of heating menstruum composition and processing conditions before and during heating were tested in a multiple regression model. In the first model (R^2 = 64.7%, s.e. = 0.539), the logD values were only significantly affected by a_w, sugar (% ww), pH, glycerol (% ww), whether cells were washed between growth and heating ("cell wash") and the duration of storage between growth and heating. Fat and other pretreatment conditions had no significant additional effect in this data set. Subsequently, the effect of temperature was found to significantly interact with water activity and pH, which resulted in a model with these multiplicative terms, sugar and cell wash, but without glycerol and storage time (R^2 = 75.1%, s.e. = 0.453). Entering serovar (n = 27) as a variable resulted in further improvement (R^2 = 78.4%, s.e. 0.422), but most serovars are not significantly different from each other. As the serovar in raw material is not controllable, it is not a useful part of a predictive model.

CONCLUSIONS AND IMPACT OF THE STUDY

This study shows that adjusting for the effects of water activity, pH and sugar content results in a model that can be used to predict the (variability of) heat inactivation of *Salmonella* serovars in liquid foods.

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Parameter estimation for molds growth on solidified apple juice from isothermal and optimal non-isothermal experiments

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OBJECTIVE

The usual experimental approach in predictive microbiology consists of performing isothermal experiments defined by factorial design, evaluating the microbial growth under the different conditions. However, isothermal experiments lead to model non-identifiability problems [1]. The Optimal Experimental Design for Parameter Estimation (OED/PE) method has been used as an alternative to factorial design, due to the improvement of model identifiability and reduction of experimental workload (and costs). It is achieved by simplifying the experimental planning through optimizing dynamic experiments [4]. *Byssochlamys fulva* and *Neosartorya fischeri* have been reported as spoilage agents and as mycotoxin producers in apple juice, thus they have great importance in food safety. The objective of this study was to estimate the growth parameters for *B. fulva* and *N. fischeri* on solidified apple juice from isothermal and optimal non-isothermal experiments.

METHODS

B. fulva IOC (*Instituto* Oswaldo Cruz) 4518 strain was isolated from apple juice concentrate, and *N. fischeri* was isolated from a processing line of apple nectar. The solidified apple juice (12 °Brix) was prepared from concentrated apple juice (70 °Brix) added by water and 1.5% of agar. The molds were inoculated in the growth medium previously placed in Petri dishes. The evaluation of microbial growth was done by measuring the colony diameter (mm) over time (days). The isothermal experiments were conducted at 10, 15, 20, 25, and 30 °C, and the non-isothermal experiments were optimally designed. *B. fulva* and *N. fischeri* growth curves were described by Baranyi and Roberts primary model [3], and the maximum growth rates by Square Root secondary model [5]. The h_0 parameter of Baranyi and Roberts model was fixed by the averaged value from isothermal experiments; the b and T_{min} parameters of Square Root model were estimated from isothermal and optimal non-isothermal experiments. The mathematical simulations were performed using Matlab software with Curve Fitting toolbox and AMIGO toolbox [2]. The nominal parameters were based on parameters from isothermal experiments.

RESULTS

The estimated model parameters (and confidence regions of the estimated parameters, in parenthesis) from isothermal experiments were h_0 = 53.42 (± 33.11), b = 0.224 (± 0.031) $day^{0.5}$ $°C^{-1}$ and T_{min} = 4.96 (± 2.30) °C for *B. fulva*, and h_0 = 35.40 (± 9.32), b = 0.194 (± 0.035) $day^{0.5}$ $°C^{-1}$ and T_{min} = 6.46 (± 2.76) °C for *N. fischeri*. For the non-isothermal experiments, some temperature profiles were tested, for which the step-wise profiles produced the most informative experiments, which corroborate the findings reported by the literature [1, 4]. Then, the optimal experiment was designed starting at 10 °C, with steps of 1.5 °C every 1 day, and experimental duration of 15 days for *B. fulva* and 20 days for *N. fischeri*.

The estimated model parameters from optimal non-isothermal experiments were b = 0.205 (± 0.007) $day^{0,5}$ $°C^{-1}$ and T_{min} = 3.38 (± 0.60) °C for *B. fulva*, and b = 0.238 (± 0.005) $day^{0,5}$ $°C^{-1}$ and T_{min} = 11.52 (± 0.28) for *N. fischeri*. The confidence regions of the estimated parameters from optimal non-isothermal experiment were smaller than those obtained from isothermal conditions, for both molds. However, the estimated parameters for *N. fischeri* were not as close to the nominal parameters as wanted. The uncertainty on the estimated parameters can be related with the high model sensitivity to experimental noise and model parameter correlation [6].

CONCLUSIONS AND IMPACT OF THE STUDY

The parameter estimation for molds growth on solidified apple juice from isothermal and optimal non-isothermal experiments produced satisfactory results, and these results can be improved with further experiments. In conclusion, the OED/PE method can be a powerful and useful tool to obtain the parameters for molds growth in foods from reduced number of experiments.

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Critical assessment of the Time-To-Detection method's performance to estimate microbial growth parameters

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OBJECTIVE(S)

Viable plate counting is the most accurate and widely used method for microbial growth parameters' estimation (mu_{max}, lag phase); however, it is laborious and time consuming. Therefore, it is of high importance to develop techniques equally reliable, able to minimise the experimental load. Time-To-Detection (TTD) method, based on turbidity measurements, was developed to fulfill these requirements (Cuppers et al. 1993). This method is widely applied, but its performance is contradictory in literature. A sensitivity analysis of the factors influencing the method is necessary to define the boundaries of reliable performance.

METHOD(S)

Three *Listeria monocytogenes* strains (LMG 23775, LMG 23905 and LMG 21263) were selected from the BCCM/LMG bacteria collection of Gent University (Belgium). A full factorial experimental design with different temperatures (4, 8, 12°C), pH values (6.0, 6.4, 6.8), NaCl concentrations (1.9, 2.6, 3.3%) and atmospheres (aerobic and vacuum) was implemented in Brain Heart Infusion broth (BHI), based on intrinsic and extrinsic conditions found in real food products. Optical density (OD) measurements (595 nm) were taken at regular time intervals with VersaMax™ Absorbance Microplate Reader. Microbial growth parameters were estimated by using the TTD method and a detailed analysis of the different variables affecting its performance was performed. Serial decimal dilutions (N_0 values) from 10^5 to 10^0 cfu/mL were used. A threshold value of optical density was set (0.2) and the time required for each of the inoculum levels to reach this threshold is plotted as a function of $\ln(N_{turb}/N_0)$, forming a linear regression. N_{turb} is the cell population when OD is 0.2. The inverse of the slope of the regression line equals mu_{max} and the intercept with the y-axis equals the lag.

RESULTS

Experimental results obtained illustrate that the TTD method struggles to describe the occurring phenomena and accurately estimate the growth parameters under realistic food-related scenarios. The relationship between TTD and $\ln(N_{turb}/N_0)$ deviates from the typical linear regression when N_0 decreases and the stress increases, resulting in negative lag estimation and underestimation of mu_{max}. Similar behavior is observed in literature (Robinson et al. 2001), while in Mytilinaios et al. (2012) TTD's performance is successful under optimal conditions and low N_0. The method depends on the variables N_0, N_{turb} and TTD, which are all subject to uncertainty and variability. The selected N_0 range (and the dilution error) has an effect on the output of the method, as previously described. N_{turb} depends on the accuracy of the calibration curve relating OD and viable counts, and the OD threshold selected as the detection limit. For this reason, the Total Least Squares regression technique was applied in order to account for errors of both dependent and independent variables of the calibration curve. By changing the range of OD included in the calibration curve the output was evaluated. Finally, the TTD is estimated with different available models (i.e., interpolation function, Baranyi (1994) or Richards (1959)); the output of the method is significantly influenced by the model selected.

CONCLUSIONS AND IMPACT OF THE STUDY

The TTD method has limitations to precisely estimate the growth parameters of *L. monocytogenes* under stressing conditions and low inoculum levels as occurring in real food products. The results ofthis study have significant implications for estimating parameters relevant in food safety assurance systems.

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PREDICTIVE MODELS FOR FOOD QUALITY AND SAFETY

Oral conferences

Biofilms structural dynamics and pathogens persistence

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In natural, industrial and medical environments, microorganisms mainly live as structured and organized matrix-encased communities known as biofilms. In these communities, microorganisms demonstrate coordinated behaviour and are able to perform specific functions such as dramatic tolerance to antimicrobials that potentially leads to major public health and industrial problems. It is now recognized that the apparition of such specific biofilm functions is intimately related to the three-dimensional organization of the biological edifice, and results from multifactorial processes. The functional properties of biofilms therefore result from the complex spatial and temporal differentiation of cells in the dynamic three-dimensional structure in response to environmental signals and cell-cell interactions. The development of tools enabling in-situ observation of dynamic processes within biofilms is required to improve our understanding of biofilm traits and to develop advanced control strategies. The recent developments in non-invasive confocal laser scanning microscopy (CLSM), coupled with the emergence of new fluorescent reporters allowing the labeling of specific matrix components or cell states have deepened our knowledge of biofilms properties and sociobiology. Non-invasive 4D microscopic approaches also provide experimental data needed to implement "virtual labs" such as spatially explicit individual based model that are now considered in food predictive microbiology.

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Romain Briandet is the leader of the biofilm team at the French Micalis Institute. Micalis is a joint research unit of ~350 persons associating INRA and AgroParisTech with the aim of developing innovative research in the field of Food and Gut Microbiology for Human Health. Romain Briandet has focused his research on the microbial biofilms present in the food chain with special emphasis on their role in the persistence of pathogens. One of his main scientific lines of interest is to identify the link between the spatial organisation of biofilms and the survival mechanisms of cells facing harsh environments such as the exposition of antimicrobials. For that, he has developed expertise on multimodal fluorescence imaging using confocal laser scanning microscopy, including spatio-temporal approaches.

Impact of line design and process characteristics on potential biofilm recontamination of an aseptic-UHT process line

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OBJECTIVE

A Quantitative Microbial Exposure Assessment (QMEA) model has been developed and applied to aseptic Ultra-High-Temperature (UHT)-type processed food products (Pujol et al., 2012). The aim of the model was to quantify the microbial contamination associated to an aseptic-UHT line, and consequently assess the commercial sterility failure rate. The model outputs were the number of packs contaminated, and when contaminated, the number of microorganisms per pack.

An initial sensitivity analysis (Mokhtari et al., 2005) revealed that biofilm contamination was a key contributor to the sterility failure rate, a result which is in agreement with generally accepted tenets of hygienic design (Marchand et al., 2012).

The objective of this study was to further refine the model by describing the impact of line design and process characteristics on the potential for biofilm recontamination of an aseptic-UHT process line.

METHODS

In our QMEA model, aseptic-UHT production line has been analyzed and then broken down into five contamination pathways: (1) microbial introduction through raw materials, (2) survival after heat-treatment or sterilization processes, (3) recontamination through air, (4) recontamination through biofilm formation on equipment surfaces, and (5) microbial growth. These contamination pathways have been applied to both food product and packaging.

The model incorporated knowledge from food engineering science, predictive microbiology and probability theory (Pujol et al., 2013). The model was set up with probabilistic inputs. It assessed the sterile failure rate for four bacteria: three spore-forming and one vegetative. The four bacteria may form or attach on equipment surfaces which then can result in biofilm formation.

The initial model describing the recontamination through biofilm formation was refined as follows. In a first step, information was collected from expert elicitation sessions organized by phone conference, questionnaire or factory visits. In a second step, the corresponding expert knowledge was turned into probability distribution function inputs. Finally, these input distributions were validated with the contributing expert.

RESULTS

The most difficult inputs to quantify were the microbial transfer between the contaminated product to the surface (the initial step of biofilm formation) and between the contaminated surfaces to the product (biofilm release leading to recontamination). Through the expert elicitation sessions, these variables were correlated to the aseptic-UHT equipment design (e.g. valve number, pipe diameter change) and to the process (e.g.

Reynolds number, shear force). First of all, the influence of the equipment design and process factors was ranked.

Then, the impact of the most influential factors was introduced into the QMEA model as an additional equation. The model parameters were set as Pert distributions to integrate their variability and uncertainty through the experts' responses. This QMEA model still indicated that biofilm recontamination is a key source of sterility failure risk and provided more accurate outputs with which to now compare different production line configurations and suggest options to reduce sterility failure rates.

CONCLUSIONS AND IMPACT OF THE STUDY

The aseptic-UHT line design and process characteristics on potential biofilm contamination have been quantified and introduced into an aseptic-UHT QMEA model through expert elicitation. This model could be used to optimize aseptic-UHT line settings and design, and to contribute in the risk management of commercial sterility failures.

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Modelling the combination of the effect of a heat treatment, temperature and medium on the individual cell lag times of Cronobacter spp.

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OBJECTIVES

Cronobacter spp. are increasingly regarded as emerging opportunistic pathogens and have been associated with outbreaks of meningitis, sepsis and necrotizing enterocolitis among infants following consumption of powdered infant formula (PIF) (Cawthorn et al. 2008). The ability of *Cronobacter* to grow at 5.5°C makes these microorganisms particularly important, as they may grow in refrigerated food during their shelf life (Nazarowec-White & Farber 1997). Since PIF is not sterile (Farber 2004), it is recommended by FAO/WHO (2006) to reconstitute it in water at 70°C to reduce bacterial load. However, this recommendation is not always followed and PIF may have instructions showing reconstitution temperature as low as 40°C (Forsythe 2009). The purpose of this study was to measure and model the effect of the variability of lag times and growth of low numbers of sub-lethally heat stressed *Cronobacter* subsequently grown at sub-optimal temperatures in tryptone soy broth (TSB) and PIF to improve risk assessments.

METHODS

The potential ability for damaged individual cells to self-repair and subsequently initiate growth in TSB at 7°C, 12°C and 22°C was investigated using optical density (OD) measurements after heat treatment at 49°C for 7 min. (Métris et al. 2003). The cells were diluted to obtain on average one cell per well and the time for each individual cell to reach a given detection level estimated. The lag times were estimated from the detection times, assuming that the single cell lag times followed a shifted Gamma distribution and the number of cells per well followed a truncated Poisson distribution (Metris et al. 2008). To validate the data in PIF, the distribution of the number of cells was estimated by plate count after incubation of a low number of stressed cells in both TSB and PIF stored at 7°C, 12°C, and 22°C for different times depending on recovery temperature (Miled et al. 2011). Monte-Carlo simulations were carried out in Matlab to compare the distributions of single cell lag times and cell numbers after incubation and predict bacterial numbers after different storage times.

RESULTS

As with *Listeria*, the heat treatments induced a shift in the time during which cells were unable to divide (Metris et al. 2008), which was longer at lower incubation temperatures. After the heat stress, the coefficient of variation of the individual lag times (the lag times minus the shift) at 12 and 22°C seemed to be close to 1 and independent of the incubation temperature, suggesting that the coefficient of variation depends in this case on the stress applied earlier, rather than on the incubation temperature of the recovering cells (Guillier et al. 2005, Metris et al. 2008). At 7°C, however, the relationship between the non heat-stressed and heat stressed bacteria did not follow the same trend as at higher recovery temperatures, presumably because the cold is already a stress and combination of the heat stress and cold is not a simple additive relationship. The simulations showed that the kinetics in PIF could be extrapolated from the kinetics in TSB, so models prepared using broth may be used for risk assessments.

CONCLUSIONS AND IMPACT OF THE STUDY

Our experimental results and simulations showed that the adding water at 49°C to PIF may constitute a significant risk of *Cronobacter* spp. infections, especially if the PIF is subsequently stored. It also showed that growth from low numbers of cells can be modelled empirically using data from TSB. Finally, it highlighted that the behaviour of bacteria facing multiple stresses (heat treatment and low incubation temperature in this case) needs to be studied further, possibly at a systems level, to improve predictions.

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Isolation, quantification and characterization of acid resistant variants of Listeria monocytogenes

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OBJECTIVE(S)

The presence of stress resistant subpopulations of *Listeria monocytogenes* is a major concern for the food industry because it leads to tailing of the inactivation curves and a higher number of surviving microorganisms than expected [1]. *L. monocytogenes* may be exposed to acidic conditions in foods and during gastric passage, indicating the importance of acid stress survival capacity. Acid resistant subpopulations would be more likely to survive stomach passage and potentially cause disease. The objective of this study was to determine if acid exposure leads to selection of resistant subpopulations and to isolate, quantify and characterize acid resistant variants.

METHODS

Late-exponential phase cells of *L. monocytogenes* LO28 were exposed to pH 2.5, 3.0 and 3.5 and a biphasic model with shoulder was used to describe the inactivation data [2, 3]. 100 colonies, isolated after 90 minutes at pH 3.5, were repeatedly sub-cultured and retested for acid resistance. The isolates exhibiting increased resistance compared to the WT were considered resistant variants. The inactivation kinetics of the acid resistant variants were also fitted with the biphasic model and a stepwise approach was followed to reduce the number of model parameters allowing us to compare the inactivation kinetics of the WT and the variants. The growth rate of the variants was determined in BHI at neutral pH and at pH 5.0. Also, the growth and inactivation at the growth-no-growth boundary (pH 4.2-4.5) was studied, and a reparameterized Weibull model was used to estimate the time to 3 decimal reductions (t_{3D}), allowing a quantitative comparison of the different variants.

RESULTS

The inactivation curves of LO28 at pH 2.5, 3.0 and 3.5 showed considerable tailing. The biphasic model with shoulder described the data well, and by using a log-scale, the significance of the parameter for the resistant fraction could be better estimated. Acid resistant variants were isolated from the tail of pH 3.5 and these variants were characterized by a highly increased resistance to pH 2.5 in both late-exponential and stationary phase. The inactivation profile of the variants at severe pH stress could be described by a linear inactivation model. While large differences in acid resistance between the WT and a group of variants were observed at severe pH stress, no differences were found in the growth boundaries and acid resistance of the WT and the variants at pH 4.2–4.5. Also, acid resistance could be correlated to a slightly reduced growth rate in BHI. The results indicate that the variants have a significantly better chance of survival at severe pH stress but this resistance does not relate to better growth or survival at conditions around the growth-no-growth boundary.

CONCLUSIONS AND IMPACT OF THE STUDY

This study shows that acid exposure of late-exponential phase cells reveals the presence of acid resistant subpopulations and that there is a phenotypic diversity amongst them. The occurrence of such acid-resistant variants may increase the chances that even low numbers of microorganisms present in food products will pass the stomach and subsequently cause infection in humans. This group of variants extends the group of previously isolated variants from different types of stress and underlines the importance of gaining more insight in the mechanisms underlying this heterogeneity and increased resistance.

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Single-cell-based conditional growth model to describe the effect of medium on the survival and growth of Salmonella under osmotic stress

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OBJECTIVES

In predictive microbiology, models are either built from measurements in specific foods or in culture medium. The generated predictions are then extrapolated to the food systems in question. This is simple and efficient if there is a constant bias factor between the growth rates in culture medium and food (Ross 1996, Baranyi et al. 1999). Whilst this has been demonstrated for growth rate, no such relationship has been studied in the case of the physiological state of the cell. The objective of this study was to evaluate the physiological state of single cells of *Salmonella* under osmotic stress in different media and extrapolate the population growth from one medium to another.

METHODS

Cultures of *S. enterica* Typhimurium in both Luria-Bertani (LB) and Basic Minimal Medium (BMM, Zhou et al. 2011) were diluted to obtain a gradient of concentrations from ca 1 to 5 log cells/well. Growth was measured in different osmotic conditions (3.5%, 5.0%, 6.5% and 7.5% NaCl in LB; 3.5% and 5% NaCl in BMM; 3.5% and 5.0% NaCl in BMM with glycine betaine) using a Bioscreen (Labsystems), an automated optical density (OD) meter. The actual cell concentration at the different dilutions was checked by plate counts. Cultures were incubated at 37^{o}C, and the OD was measured at a wavelength of 600nm to determine the times to reach an OD of 0.2. The "physiological state" of individual cells was quantified as proposed by Baranyi and Pin (1999). The data analysis, carried out in Microsoft Excel and Mathworks Matlab, was based on their method, with a modification, in order to take into account the variability of the growth rate. The population growth curves were generated as in Pin and Baranyi (2006).

RESULTS

The single cell experiments indicated that the individual physiological states were more spread in LB than in BMM suggesting that the cells with low physiological states were unable to grow in BMM. This is in agreement with the observation of Zhou et al. (2011) that, at the population level, in BMM, the population initially declined before starting to grow, while this did not occur in LB. Simulations showed that the "late growers" in LB (the cells with a low physiological state parameter) contribute little to the population. We simulated the growth of the surviving fraction in BMM using the concept of conditional probability. The probability p was defined to link the physiological states in rich and minimal medium, values of which varied with NaCl concentration.

CONCLUSIONS AND IMPACT OF THE STUDY

Modelling the effect of the growth medium on the physiological state is not as straightforward as for the growth rate because the medium has an effect on the probability of growth as well as on the physiological state of single cells.

Our findings highlight the importance of single cell level analysis to model adaptation and survival (Guillier and Augustin, 2006, McMeekin *et al*. 2013).

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Effect of temperature shifts on germination of Penicillium chrysogenum conidia

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Germination is a key step in the development of filamentous fungi. Germination can be divided into 4 stages: activation that breaks dormancy, swelling that allows hydration of the conidia, polarized growth that leads to the creation of many local activity centers, and production of the germ tubes.

In the environment, fungal conidia are subject to transient conditions. In particular, temperature is varying according to day/night swifts. All predictive models of the germination time assume that fungal spores can adapt instantaneously to changes of temperature. The only study that supports this assumption (Gougouli et Koutsoumanis, 2012) was carried out on *Penicillium expansum* and *Aspergillus niger* conidia that already produced germ tubes.

In contrast, the present study focuses on temperature shifts applied during the first stages of germination (i.e., before the apparition of the germ tubes). Firstly, germination times were determined in steady state conditions at 10, 15, 20 et 25°C. Secondly, temperature shifts (e.g., up-shifts and down-shifts) were applied at 1/4, 1/2, and 3/4 of these respective germination times. The magnitude of the shifts was 5, 10 et 15°C.

Experiments were carried out in triplicate on *Penicillium chrysogenum* conidia on Potato Dextrose Agar medium according to a full factorial design. Statistical analysis of the results clearly demonstrated that the assumption of instantaneous adaptation of the conidia should be rejected. Temperature shifts during germination led to an induced lag time or an extended germination time as compared to the experiments conducted ay steady state. The induced lag time was maximized when the amplitude of the shift was equal to 10°C. Interaction between the moment and the variation (upward or downward) of the shift was highlighted. The induced lag time for a 10°C down-shift applied at 1/4 of the germination time was significantly less than that observed for the up-shift disturbance. These results suggest that germination is not a linear process, but that mechanisms responsible for the first stages of the germination are faster than the latter ones.

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Combining growth/no growth models for osmophilic yeast and xerophilic molds to predict the stability of IMF

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OBJECTIVE(S)

The microbial stability of intermediate moisture foods (IMF) is linked with the possible growth of three specific spoilage organisms: osmophilic yeasts (*Z. rouxii*) as well as xerophilic molds (*W. sebi* and *E. herbariorum*). Therefore, it is important that microbial stability models for these specific spoilage organisms can be combined. For products with a long shelf-life such as IMF growth/no growth models are of utmost importance for the assessment of the microbial stability and for the product innovation. Next to environmental stress factors also time was incorporated as an explanatory variable in the model for the yeast.

METHODS(S)

Sabouraud broth and malt yeast extract with high sugar concentration were used as specific growth medium for yeast and molds, respectively. Media differed in pH (5.0 -6.2, 4 levels), a_w (0.76 – 0.88, 4 levels), ethanol concentration (0 – 14.5% on water phase, 4 levels) and acetic acid concentration (0 – 3% on water phase, 4 levels). Inoculation was performed at a level of 4 log CFU/ml for yeast and 4 log spores/200 µl for molds. Growth at 22°C was followed during > 60 days for yeast and > 90 days for molds by OD measurements. Logistic regression based models with pH, a_w, ethanol, acetic acid and time as variables were fitted to the data. Detailed description of the methods can be found in Vermeulen et al. 2012 and Deschuyffeleer et al., *in preparation*)

RESULTS

Results proved that *Z. rouxii* was not highly inhibited by low pH's (2.5), ethanol concentrations until 6-9%, a_w > 0.75 and the presence of acetic acid. Therefore, a combination of these stress factors must be applied. To quantify the combined effect, a G/NG model was developed which also included incubation time. The models showed that the G/NG interface only changed minorly between 40 and 60 days of incubation at room temperature, implying that for the development of models for IMF products with long shelf-lives a limited experimental time suffices to determine the G/NG interface. The added value of incorporating time as an explanatory variable was limited. The advantage of predicting the growth/no growth boundary at specific times will be negated by the significant enlargement of this boundary, leading to a less accurate prediction of the growth/no growth interface. For xerophilic molds the same stabilisation of the G/NG interface as a function of time was observed, although later than for yeast (> 90 days). Results showed that the growth of *W. sebi* and *E. herbariorum* can be inhibited for a prolonged time (> 3 months) if an ethanol concentration of 5% on the water phase is present in the food product, irrespective of water activity values between 0.89 and 0.755. The necessary amount of ethanol for shorter shelf lives can be calculated with the models that were build. On the other hand, the growth of *W. sebi* was clearly stimulated by low ethanol concentrations (1.5%-2.0% on water phase) which complicated the model predictions. For the molds, acetic acid was not included in the models because a screening experiment revealed a high sensitivity for even low concentrations (0.5% w/w, about 1.0 % on the water phase).

CONCLUSIONS AND IMPACT OF THE STUDY

Growth/no growth models which combine the growth of osmophilic yeast and xerophilic molds allow to estimate the microbial stability of intermediate moisture foods with typically a long shelf-life. This will stimulate the innovation in these type of products.

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Variation in the growth/no growth interfaces among 189 Escherichia coli strains from different sources as a function of temperature and pH adjusted with HCl and lactic acid

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OBJECTIVE(S)

The heterogeneity of the growth/no growth (G/NG) response among 189 *Escherichia coli* strains was assessed at all combinations of 3 temperatures and 6 pH values adjusted with HCl and – in a second set of experiments – lactic acid (LA). The objectives of this study were: (i) to evaluate how large is the variation in the minimal pH allowing growth for a large number of *E. coli*, (ii) to model the G/NG interface as a function of pH, undissociated lactic acid concentration and temperature using logistic regression models, (iii) to draw a zone of growth probability for *E. coli*, and (iv) to put the differences in growth responses into perspective given existing literature on G/NG modeling.

METHODS(S)

97 strains were isolated from different sources in the environment. The others include APEC, ECOR, CNF-producing, ETEC, EPEC, ATCC, NTEC and MG1655. Stationary-phase inocula were prepared by inoculating a single colony in Luria-Bertani media (LB) in a 96-well microplates (MTP) incubated at 37˚C for 18h to yield a cell concentration of approximately 10^9 CFU/ml. Four replicates were performed. Appropriate dilutions of the stationary-phase inocula were transferred to LB with adjusted pH, the final volume was 200µl and the initial cell concentration was approximately 105 CFU/ml. The factors examined were temperature (20, 25 and 30˚C), pH adjusted with HCl (3.8, 3.9, 4.0, 4.1, 4.2 and 4.3) and with LA (4.4, 4.5, 4.6, 4.7, 4.8, 4.9). LA was added to LB media to reach the final total concentration of 25mM, afterwards pH was adjusted with HCl and NaOH. All MTP were covered with a special sandwich cover to prevent evaporation and ensure homogeneous distribution of oxygen. The MTP were placed in clamps and incubated at the specified temperature under agitation at 200rpm. After 3 days the optical densities (OD) of the MTP were recorded at 600nm. Growth was considered when the OD value reached 0.15 units [1].

RESULTS

For the screening with HCl, the minimum pH allowing growth varied significantly going from the lowest to the highest pH tested at 20 and 25˚C, and at 30˚C from 3.8 to 4.2. One of the most resistant strains, isolated from minced meat, grew in 50% of the replicates at 20˚C and pH 3.8, while the bovine necrotoxigenic *E. coli* (NTEC) showed 50% of growth at 20˚C only at pH 4.3. The transition from growth to no growth zone occurred gradually for all strains tested. The interface between growth and no growth is usually abrupt [2]. It is possible that the short time of analysis (3 days) resulted in gradual transition. However, McKellar et al. (2001) [2] observed growth likewise after 3 days and in their analysis the interface between G/NG was abrupt. In all but one case an adequate logistic regression model was identified. The experiments with lactic acid are currently being conducted.

CONCLUSIONS AND IMPACT OF THE STUDY

Checking the variability in acid resistance within a large number of *E. coli* strains is of great importance to understand the behavior of possible emergent tolerant strains that are not normally tested in literature. The first screening with HCl showed that the minimal pH allowing growth for *E. coli* could be considered as a zone and not as a unique value. This study will provide a broad knowledge in the G/NG zone of a representative number of *E. coli*. We will also put the obtained minimal pH values for growth in perspective considering existing literature results on G/NG modeling and associated parameter estimation.

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Modelling quality of frozen fish products displayed and sold chilled and selection of Time Temperature Integrators to monitor their shelf life

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OBJECTIVE

Application of an optimized quality and safety assurance system for the chilled and frozen distribution of products requires continuous monitoring and control of storage conditions, from production to consumption. Time-Temperature Integrators (TTI) are active "smart labels" that show an easily measurable, time-temperature dependent change that reflecting the temperature history of the food product. Certain fish products are produced, packed, stored and distributed frozen and are displayed and sold at the retail level refrigerated after controlled thawing. Shelf life after thawing depends on the conditions during frozen storage (that can extend for several months) as well as the chilled storage conditions (several days) up to the moment of consumption. The objective was to evaluate the applicability of appropriate photochromic and enzymatic TTIs for monitoring the quality of frozen blueshark slices that are thawed and sold chilled.

METHODS

Frozen blueshark slices (*Prionace glauca*) were packed and stored at isothermal conditions (-15 to 15°C). Quality was modelled based on microbial growth (during chilled storage), TBARs, TVB-N and sensory scores. Temperature-dependence of rate constantswas modelled by the Arrhenius equation, separately for the frozen and the chilled stage. An enzymatic and a photochromic TTI were tested. The M-type enzymatic TTIs (VITSAB, Sweden) from initial green, becomes progressively yellow/orange and reaches a red colour. Response for different enzyme concentrations waw kinetically modelled. The response of the B1 photochromic OnVu^{TM} TTI (Freshpoint, Israel and Bizerba, Germany) depends on the length of UV light exposure during activation. Kinetic modelling of response was based on measurements of the response of 5 replicate TTI tags isothermally stored (from -15 to 15ºC). The response of the TTI was modelled as a function of time, temperature and initial charging conditions or enzyme concentrations.

RESULTS

Kinetic models in order to allow estimation of the quality of frozen blueshark slices in the real distribution path and then the shelf life during the subsequent chilled storage foods were developed. Shelf life at subzero temperatures was determined from the models of TVBN or sensory indices while growth models of spoilage psychrotrophs were used for chilled shelf life. Shelf life at -15 and -10ºC is 356 and 130 days respectively, while after thawing fish slices can be stored for 11 at 0 and 8 at 5ºC. To assess the application scheme and the monitoring reliability of appropriate TTI during both the frozen and the chilled stage. Photochromic TTIs activated for 0,3 sec and enzymatic TTIs of 20units of enzymatic activity were used, that correlated well with the shelf life of blueshark slices. A distribution scenario in the current cold chain was simulated. It included an initial stage of frozen storage in the packing plant, frozen transportation and storage at the distribution center. Fish was then thawed overnight at 0°C, transported as chilled and kept at retail refrigerators until being purchased by the final consumers. The extent of quality deterioration and the remaining shelf life at the end of each distribution phase was estimated by the developed fish quality models and TTI response. With

the error for the remaining shelf life indicated by TTI being less than 10%, the potential of using TTI as reliable tools for quality assessment of products at the given point of such a food distribution chain is substantiated.

CONCLUSIONS AND IMPACT OF THE STUDY

In this study a systematic modelling of quality deterioration of fish at frozen and chilled conditions was achieved. The models combined with the use of the selected TTI could be an effective tool for monitoring of the quality of frozen fish that are thawed and sold chilled.

Development and validation of an extensive stochastic model for the simultaneous growth of Listeria monocytogenes and lactic acid bacteria - A case study with naturally contaminated cold smoked Greenland halibut

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OBJECTIVE

The objective of the present study was to develop and validate a combined stochastic model for growth of *Listeria monocytogenes* and lactic acid bacteria (LAB).

METHODS

An existing deterministic model for the simultaneous growth of *L. monocytogenes* and LAB (Mejlholm and Dalgaard, 2007, 2009) was expanded into a stochastic model using the Analytica software. The stochastic model includes the effect of 12 environmental parameters as well as their interactive effects, together with the effect of microbial interactions between *L. monocytogenes* and LAB. To evaluate the performance of the stochastic model, a total of 24 storage trials with naturally contaminated cold-smoked Greenland halibut (CSGH) was carried out at 5, 8 and 12 °C. Samples were supplied by a Danish seafood processer from a withheld batch of CSGH being positive for *L. monocytogenes.* The variability in product characteristics was determined by analysis of five samples. In addition, 56 samples of CSGH were collected from the same company during a period of 12 months to obtain a more thorough description of the variability in product characteristics and in the occurrence of *L. monocytogenes* and LAB. In contrast to traditionally produced CSGH, all the examined samples were added acetic and lactic acids in order to improve the safety of the product.

RESULTS

Product characteristics of CSGH used for the storage trials were: Water phase salt (3.31 ± 0.41%), pH (6.13 ± 0.15), phenol (10.1 ± 0.8 ppm), water phase acetic acid (3011 ± 472 ppm) and water phase lactic acid (7657 ± 1162 ppm). The initial concentration of *L. monocytogenes* in CSGH was 0.13 ± 0.28 log (cfu/g), with all samples being positive. No growth of *L. monocytogenes* was observed at 5°C whereas an increase in the concentration was seen for some of the storage trials at 8 and 12°C, with the maximum population density (MPD) of the pathogen reaching approx. 1.7 log (cfu/g) at both temperatures. By including a relative lag time (RLT) of 4.5 for *L. monocytogenes* (Ross, 1999), MPDs of 1.3, 1.6 and 3.2 log (cfu/g) were predicted by the stochastic model at 5, 8 and 12°C, respectively. Without a RLT of 4.5, the corresponding predictions were 2.8, 3.6 and 4.2 log (cfu/g). Product characteristics of the 56 samples of CSGH were: Water phase salt (3.10 ± 0.53%), pH (6.12 ± 0.16), phenol (10.4 ± 4.2 ppm), water phase acetic acid (3586 ± 1061 ppm) and water phase lactic acid (9701 ± 1954 ppm). Assuming a shelf life of 28 days at 5 °C, *L. monocytogenes* was predicted to grow to no more than 1.5 log (cfu/g) in CSGH. Without a RLT of 4.5, the corresponding prediction was 3.4 log (cfu/g). Predicting growth of *L. monocytogenes* in CSGH without addition of acetic and lactic acids resulted in a MPD of 3.3 log (cfu/g) and more than 25% of the samples were estimated to exceed the regulatory limit of 2.0 log (cfu/g).

CONCLUSIONS AND IMPACT OF THE STUDY

It was clearly demonstrated that to accurately predict the MPD of *L. monocytogenes* in naturally contaminated CSGH both the lag time and the effect of microbial interaction needs to included. Without

these effects the MPD of *L. monocytogenes* was predicted to be up to 100.000 times higher than observed and high percentages of samples were estimated to exceed 2.0 log (cfu/g). Furthermore, it was shown that CSGH with added acetic and lactic acids complied with the EU-regulation (EC 2073/2005) on *L. monocytogenes*, even when the variability in product characteristics was taken into account. Without the addition of acetic and lactic acids, CSGH constitutes a high-risk product with the potential of causing listeriosis.

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Extending the gamma concept to non-thermal inactivation: an innovative model to predict the fate of Salmonella during the dried sausages process

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OBJECTIVES

The process of dried sausages includes an acidification step during which the starter culture decreases the pH from 6.0 to 4.5, followed by a drying step to reach an a_w of 0.85 at a temperature of 12-15°C. These conditions are usually not favorable to the development of *Salmonella*, and a reduction of the *Salmonella* population is generally observed. However, this zoonotic agent was involved in two foodborne outbreaks in France in 2010 (132 cases) and 2011 (337 cases) where the consumption of dried sausages was strongly suspected (Gossner *et al.*, 2012). The objective of this study was to develop a model describing the evolution of *Salmonella* during the fabrication process of dried sausages and to optimize the food formulation to prevent the pathogen presence the end of the process.

METHODS

An experimental design was set to investigate the effects of the process for several formulations, taking into account the type of starter culture (fast growth rate or moderate growth rate starter), the sodium chloride concentration (24g/kg or 28g/kg), the dextrose concentration (5g/kg or 8g/kg) and lactose concentration (0g/kg or 10g/kg). For each condition, raw material were artificially contaminated with a *Salmonella* Thyphimurium strain at a 2 log CFU/g concentration before they undergo the steaming and drying process. At pre-established dates, samples were collected, and the following analyses were performed: *Salmonella* and lactic acid bacteria enumeration, pH, aw, loss of weight and lactic acid concentration. The growth/inactivation model based on the gamma concept (Coroller *et al.*, 2012) was then adapted to describe the *Salmonella* behaviour in dynamic environmental conditions. For the used *Salmonella* strain, the growth and inactivation parameters for temperature, pH, water activity, and lactic acid concentration were already available.

RESULTS

The growth rate in dried sausages at optimal conditions (μ_{opt}) and the bacterial resistance in reference conditions (δ^*) were estimated from the kinetical behavior of *Salmonella* and the dynamic profiles of temperature, pH, water activity and acid lactic concentration. The model allowed an accurate description of the *Salmonella* fate with a first step of bacterial growth followed by an inactivation step. During the first phase, the temperature and the water activity were the most limiting factors for the *Salmonella* growth. At the second day of the process, the decrease of temperature combined with the decrease of pH, the decrease of the water activity and the increase of lactic acid produced by the starters led to *Salmonella* inactivation. The transition between growth and inactivation was accurately described by the model with less than 12 hours of root mean squared error. Using fast growth rate or moderate growth rate starters, the *Salmonella* behaviour (μ_{opt} and δ^*) were influenced by the initialsugar concentrations but not by sodium chloride content. The growth and the resistance of *Salmonella* were higher when using a moderate growth rate starter. These differences in the *Salmonella* behavior should be correlated to differences in the starter behavior. A tool was

proposed and the predictions were validated by 8 kinetics. The mean prediction error was 1.39 logUFC/g.

CONCLUSIONS AND IMPACT OF THE STUDY

A novel growth/inactivation model was developed in this study and was able to accurately describe the behavior of *Salmonella* during the dried sausages process taking various processing factors into account. Moreover, this study showed that the cardinal values and inactivation parameters acquired in static conditions can be used to predict bacterial fate in dynamic environmental conditions. This study therefore represents a breakthrough in the field of predictive microbiology applied to food processing.

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Modelling and predicting the simultaneous growth of Listeria monocytogenes and starter cultures in cottage cheese and other fresh dairy products

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INTRODUCTION

Cottage cheese is a ready-to-eat, high-moisture, fresh cheese with pH of 5.10 – 5.45 and low initial concentrations of NaCl and organic acids. Cottage cheese consists of cheese curd mixed with a cultured or uncultured cream dressing. The cheese curd with pH 4.50-4.80 is acidified by a mesophilic lactic acid bacteria (LAB) starter culture including *Lactococcus lactis* and the cultured cream is formed by a diacetyl producing variant of *Lc. lactis*. Product characteristics and previous studies have shown that cottage cheese support growth of *L. monocytogenes*. Growth of starter LAB during storage of cottage cheese has a pronounced inhibiting effect on the growth of *L. monocytogenes* corresponding to a classical Jameson effect. Existing LAB models, however, have been unable to accurately predict growth of the mesophilic starter LAB in cottage cheese. The aim of the present study was to model and predict the simultaneous growth of starter LAB and *L. monocytogenes* in cottage cheese. The effect of the two different starter LAB cultures on growth of *L. monocytogenes* was quantified and modelled.

METHODS

Data has been collected from challenge tests (5-15˚C), storage trials (5-15˚C) and growth experiments in model systems using automated absorbance measurements (5-15˚C/7-21˚C). The inhibitory effect of the starter LAB was studied by co-culture and spent-medium experiments. Simplified cardinal parameter-type models were developed for the effect of temperature, pH, salt, lactic acid, sorbic acid and for the interaction between these environmental parameters (Le Marc *et al.* 2002; Mejlholm and Dalgaard, 2007). The inhibiting effect of the two different starter LAB on growth of *L. monocytogenes* was modelled by the Jameson-effect approach (Giménez and Dalgaard, 2004). Values of cardinal parameters (T_{min}, pH_{min}, $a_{w\ min}$, MIC-values) were estimated or obtained from previously published growth models. The performance of models was evaluated by calculation of Bias- and Accuracy-factor values from kinetics in cottage cheese and other fresh dairy products.

RESULTS

Evaluation of thirteen existing *L. monocytogenes* growth models confirmed that five of these acceptably predicted growth in cottage cheese with Bias-factor values between 0.87 and 1.43. It was therefore decided to use existing cardinal parameter values to model growth of *L. monocytogenes* in cottage cheese. New cardinal parameter values were estimated for the two starter LAB cultures used in cottage cheese. Mainly T_{min} differed from values of this parameter reported in literature that were most often below 0˚C (Devlieghere *et al.* 2000; Wijtzes *et al.* 2001; Mejlholm and Dalgaard, 2007; Leroi *et al.* 2012). T_{min} of the applied starter LAB were estimated to 2.4˚C and 3.7˚C, respectively. The developed models can predict the simultaneous growth of *L. monocytogenes* and of the two mesophilic starter LAB cultures at different product characteristic and storage condition of relevance to cottage cheese.

CONCLUSIONS AND IMPACT OF THE STUDY

At the time of packaging the concentration of starter LAB in cottage cheese is 4 to 6 log cfu/g and it is

crucial to model the simultaneous growth of these LAB and *L. monocytogenes* to be able to obtain realistic predictions of the maximum population density (N_{max}) of the pathogen. This also seems to apply to other dairy products with high initial concentrations of starter LAB. Furthermore, the present study confirmed that to be accurate, predictive food microbiology models need to include the effects of several environmental parameters.

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Cardinal values: a single set to predict growth and heat resistance recovery

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OBJECTIVE(S)

The importance of *Geobacillus stearothermophilus* is well known in canned food and thermal food preservation. This flat sour spoilage organism is commonly used as biological indicator in the design of heat sterilisation process due to the high heat resistance of its spores. To control high resistant spores, the F-value (heat treatment intensity) is not the only way. Recovery medium after heat treatment significantly affects the apparent heat resistance. This work proposes to describe heat recovery using a single universal parameters set: the cardinal values.

METHODS(S)

On one hand, the influence of temperature and pH on the bacterial growth was estimated by plate count plate. At least, twelve levels of Temperature and pH were studied. The effect of temperature was studied at a constant pH (7.3), and the effect of pH was studied at a constant temperature (57.5°C). Growth rate has been estimated by fitting a primary growth model, and so cardinal values have been estimated by fitting cardinal model (Rosso *et al.*, 1995). On the other hand, spores have been produced in nutrient agar (pH 7 and 57.5°C). Apparent D-values were estimated from survival kinetics obtained at 115°C and recovered at twelve different temperatures and pHs.

RESULTS

Growth of *G. stearothermophilus* ATCC 12980 was observed between 40 and 63 ° C. Growth at 65°C was observed for one on three replicates. Based on experimental results, growth cardinal values were estimated at 38.7, 57.5 and 65.4° C respectively for T_{min}, T_{opt} and T_{max} by fitting the cardinal model. Moreover, growth was observed between 5.72 and 8.48. pH cardinal values were estimated at 5.60, 7.08 and 8.85 respectively for pH_{min}, pH_{opt} and pH_{max}. D-values obtained at different temperature and pH recovery conditions shows that the highest numbers of survivors are obtained in optimal growth conditions and the apparent D-values decreased at suboptimal pHs and temperatures. These current observations suggest the existence of an optimum recovery medium corresponding to the optimum for growth. Finally, a model was proposed to describe heat recovery using growth cardinal values. The major interest of this model is to be applicable without new parameters. This model joins the concept developed by Baril *et al.*, (2012), where cardinal values can explain many physiological characters, such as sporulation limits.

CONCLUSIONS AND IMPACT OF THE STUDY

Temperature and pH cardinal growth models were validated on a thermophilic strain. The developed model quantifies recovery medium effect on spores' heat resistance and is well-designed for canned industries.

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A systematic multivariate approach in modeling the shelf-life of fresh-cut salads as a function of temperature and packaging atmosphere

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OBJECTIVE

Fresh-cut salads have become increasingly popular over the last decades. Quality deterioration of these foods is the outcome of combined microbiological-dependent and independent changes during storage, which may represent a vector of quality and spoilage indicators. Spoilage is often associated with epiphytic microflora comprised of pseudomonads, lactic acid bacteria (LAB), Enterobacteriaceae, and yeasts-moulds, while quality degradation may also be caused by browning and enzymatic softening. The relative level and dominance of each microbial group strongly depends on vegetable decontamination (chemical washing), its innate post-harvest physiology (e.g., respiration rate), packaging atmosphere and storage conditions. The use of appropriate modified atmosphere packaging (MAP) can maintain their quality beyond the typical well-established commercial shelf-life of 7-8 days. An extensive literature review (organized in a systematic database; www.SOPHY-project.eu) highlighted the need for mathematical models that effectively describe the quality changes of RTE fresh-cut vegetables and predict their shelf-life based on packaging and storage conditions. The objective of this work was to develop kinetic and multivariate mathematical models, which integrate microbiological, enzymatic, texture, multi-spectra image analysis, and FT/IR data: (i) to identify the dominant mechanism of quality deterioration of various fresh-cut salads as a function of initial packaging atmosphere and temperature, (ii) to determine the proper packaging atmosphere mechanisms that control the dominance of aerobic or microaerophilic organisms, affecting the perception of spoilage type and intensity.

METHODS

Shelf-life tests were performed for rocket, iceberg lettuce, and cabbage from a typical production line. Samples were packed in different O_2/CO_2 ratio atmospheres (0-20% O_2/20-0% CO_2/80% N_2) and stored at isothermal (0-15°C) conditions. Total viable counts (starting at 4-5 Log CFU/g), pseudomonads, LAB, Enterobacteriaceae, and yeasts-moulds, colour (*L*, *a*, *b*), texture, enzymatic browning, vitamin loss, sensory attributes, and gas concentrations were monitored during storage. Multi-spectra imaging of vegetables was also performed, using VideometerLab (a colour and texture measurement vision system) and FT/IR spectroscopy. Kinetic modeling was based on quality estimation at appropriate time intervals. Principal Component Analysis and Partial Least Square Regression were applied for classifying products according to their major spoilage mechanism in response to packaging and temperature conditions. The models of microbial spoilage developed from the isothermal studies were validated at dynamic temperature conditions.

RESULTS

Experimental data for the measured indices was adequately fitted to first or zero order kinetics. The effect of temperature on shelf-life was expressed by activation energy (E_a) values, of the Arrhenius plots, ranging from 60 to 80 kJ/mol. The primary quality decay mechanism varied with the salad. In rocket, the texture breakdown attributable to pectinolytic enzymes activity, and modeled as zero-order decay, in parallel to loss of vitamin C, preceded microbial spoilage. In lettuce, browning of the cut edges, resulting in drop of *L* and

increase of *a* and *b* colour parameters, concurred with increase in microbial numbers. Based on microbiological data, a critical initial O_2/CO_2 ratio in MAP could be identified for each salad that determined the dominance between psychrotrophic aerobic (pseudomonads) and microaerophilic (LAB) flora. Shifting between these two groups markedly impacted the type of spoilage perceived by sensory analysis. FT/IR and multi-spectra imaging increased the resolution in detecting quality changes and assisted in classifying products based on packaging and temperature conditions.

CONCLUSIONS AND IMPACT OF THE STUDY

Systematic modeling of quality deterioration of MA packaged fresh-cut salads under refrigeration was undertaken. This lead to validated composite models enabling assessment of multiple quality indices of different fresh-cut salads stored at any initial ratios of O_2/CO_2 atmosphere and storage temperature. Expanding and/or developing shelf-life models of fresh-cut salads can serve as an effective tool in the development of new products in the fresh produce food sector.

Effect of novel food formulations on Salmonella Typhimurium growth dynamics

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OBJECTIVE(S)

Consumer demand towards low calorie food products has increased, due to a growing concern of our society on wellness and healthy nutritional habits The food industry is challenged to develop novel and healthier products , for instance, by replacing high-calorie sugars with no-caloric sweeteners (i.e., aspartame, saccharine). However, these compounds have some undesirable properties, i.e., thermolability, metallic/bitter after-taste, that lead to search for better alternatives, like stevioside and tagatose. Though, when sugar is replaced, also functional and microbial safety aspects can be affected. For instance, partial or total elimination of sugars from a food product increases its water activity (a_w), leading to a reduction in the food-intrinsic bio-preservative ability[(1)]. Dairy products are a target for low-calorie formulations[(2)] that may easily get contaminated with undesired microorganisms (*Salmonella* spp.). Available predictive models do not consider the effect of novel food formulations on microbial dynamics, which is a key factor to be addressed. In this work, the effect of novel sweeteners on the growth dynamics of *Salmonella* Typhimurium in synthetic medium and milk was assessed.

METHODS

The synthetic medium and the dairy product used in this work were Tryptone Soya Broth-dextrose-free (TSB-df) and reconstituted powder-skimmed-milk respectively. Both media were enriched with the selected sweeteners: (i) stevioside (St), (ii) tagatose (Tg), (iii) conventional sugar (S), and (iv) laboratory sucrose (LabS). Sweetener solutions in the described media were inoculated with *Salmonella enterica* serovar Typhimurium SL1344 (IFR, Norwich, UK). A stock culture was stored at -80ºC in Luria Bertani broth (LB) supplemented with 25% (v/v) glycerol. A full factorial design was implemented with different culture media (TSB-df and milk), sweeteners (St, Tg, S and LabS), their concentration levels (3, 9 and 15% (w/v)) and temperatures (8 and 20ºC). At regular time intervals, duplicate samples were taken to determine cell concentration, and the appropriate dilutions were plated on Tryptone Soya Agar (TSA). After incubation (24 h/37ºC), plates were enumerated. The obtained results were log-transformed and plotted as a function of time. The growth model of Baranyi and Roberts (1994) [(3)] was applied to estimate the growth parameters of *S.* Typhimurium.

RESULTS

Experimental results here obtained illustrate that the growth dynamics of *S.* Typhimurium are affected when sucrose in the media is substituted by stevioside or tagatose, especially in TSB-df. At 20ºC, similar growth curves are obtained between the different sweeteners, although some differences are observed when comparing the maximum growth rate (µmax). This parameter shows the highest values when the medium is enriched with stevioside at any concentration. However, opposite results are obtained with tagatose, showing always the lowest µmax values. Results at 8ºC show clear differences between sweeteners and their concentration: (i) at 3% tagatose, microbial growth is reduced, giving rise to an inhibitory effect when increasing its concentration; (ii) stevioside has the opposite effect even at high concentrations (constant N_{max}). Results obtained in milk show a similar trend, in general, although lower microbial growth is recorded for the different tested conditions.

CONCLUSIONS AND IMPACT OF THE STUDY

Results obtained in this work will contribute to select the most suitable sweeteners for the design of low calorie products. The potential effects of tagatose as antimicrobial, and stevioside as growth enhancer, should be considered for future product formulation. Implementation of this knowledge in predictive models will allow an optimal design of food safety assurance systems.

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PREDICTIVE MODELS FOR FOOD QUALITY AND SAFETY

Posters

Evaluation of antifungal activity of ethanol and methanol extracts fromPunica granatumpeels on fungal strains producing mycotoxins

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The worldwide contamination of foods and feeds with mycotoxins poses a significant health problem. Mycotoxins can cause acute or chronic intoxication and damage to humans and animals after ingestion of contaminated food and feed.

Considering these as a first step the objective of our work is to evaluate the antifungal activity of some extracts from *Punica granatum* peels on some fungal strains producing mycotoxins. Pomegranate is an ancient fruit and a known rich source of bioactive compounds. It is only recently that modern scientists have systematically evaluated the fruit for its various medicinally useful properties. In the present work four extracts obtained by an ethanol, methanol extraction and maceration from *Punica granatum* peels were tested for their antifungal potential against three species of *Aspergillus* such as *A. flavus*, *A. niger*, *A. ochraceus*, and one species of *Penicillium* such as *P. expansum*.

The assessment of antifungal activity is assayed by radial growth technique on solid medium content selected volumes of peel *Punica granatum* extracts. The assessment of mycotoxinogenesis is released on Y.E.S medium mix with selected volumes of peel *Punica granatum* extracts. The results of our work indicate that all the four extracts prevent totally the mycilial growth in solid and liquid medium with a percentage of 100% of all the fungal strains in selected concentration. Moreover the methanol extract was the more effective one and the fungal strain *A. ochraceus* was the most sensible one for all the extracts.

In conclusion the results indicate that all the extracts which have been obtained from *Punica granatum* peels got an antifungal activity against the four fungal strains.

Keywords**:** Mycotoxins, antifungal activity, *Aspergillus*, *Penicillium*, *Punica granatum peels*, methanol extract, ethanol extract.

Contribution to the study mycological and mycotoxicological some aromatic plants marketed in the south-western Algeria

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Many agricultural products are likely to contain toxigenic molds, including herbs that are often left after harvest on the ground in a dry environment, which is in most cases, the ideal conditions for mold growth and mycotoxin production. For this purpose, we contribute a mycological and mycotoxicological study for some herbs marketed in the south-western Algeria. All samples analyzed (cumin, caraway, chili, pepper and ras el hanoute) are contaminated with molds. We have identified various types of molds belonging to the flora of storage, other genre belonging to the field flora and intermediate flora. The test research of aflatoxin producing strains of differents isolates of *Aspergillus. Flavus* and *parasiticus* revealed that 50% of tested strains are producing AFB1 and AFG1 and 50% were considered non-productive.

The operating results for aflatoxins by TLC on our substrates were positive with the exception of samples Caraway has revealed negative.

Key words: moulds, mycotoxin, aflatoxin, aromatic plants

Antifungal screening of Algerian Hyoscyamus muticus overlooked mycotoxinogène strains isolated of wheat and coffee

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OBJECTIVES

The aim of this study was to test antifungal activity of aqueous and hydromethanolic extracts for *Hyoscyamus muticus* leaves and roots that grows in the region of Béchar and which has a great therapeutic importance.

METHODES

Two methods have been used with this extracts on 4 fungal strains producer of mycotoxines; determination of growth radial technique on solid medium and determination of biomass technique on liquid medium.

RESULTS

In relation to the growth of strains has shown a particular reduction in both growth , biomasses and production of mycotoxins ordered as follows: *Aspergillus Flavus, Aspergillus Ochraceus, Aspergillus Niger, Penicillium expansum* giving percentage of inhibition 100%, 61,86%, 100%, 100% for the aqueous extract and 76,08%; 100%; 18,4%; 65,38% for the hydromethnolic extract.

CONCLUSION:

Extracts of *Hyoscyamus muticus* are effective overlooked all strains mycotoxinogène tested with differantes concentrations

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Evaluating the Antibacterial Effect of Fish Protein Concentrates on Growth of Spoilage Flora in Fish Sausage on Chilled Storage

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OBJECTIVE

This work aimed to describe the effects of protein concentrates on the growth kinetics of spoilage indicators, including total bacteria, psychrotrophic bacteria, *Pseudomonas sp.*, *Staphylococcus aureus*, total anaerob, total coliform and *E. coli*, lactic acid bacteria and yeast and mould counts.

METHODS

Samples were prepared with minced fish, some spices and protein concentrates and hot smoked before vacuum packing. Protein concentrates were added at high (10%) and low (1%) levels, with a control without protein concentrates, and storage time (16 weeks). Weekly bacterial levels (log_{10} cfu/g) were determined.

RESULTS

TVC, psychrotrophic, anaerobic and lactic acid bacteria counts for all samples increased during storage time ($P<0.01$). However, *P. sp.*, *S. aureus*, total coliform and *E. coli*, yeast and mould counts were not determined during storage time.

CONCLUSIONS and IMPACT OF THE STUDY

The use of protein concentrates significantly reduced the log phase of TVC by least 1 week, and reduces the maximum final growth by 2.5 log cycles. There was a negative correlation between the rate of protein concentration and TVC, psychrotrophic, anaerobic and lactic acid bacteria counts for all samples during storage time ($P<0.01$). The use of protein concentrates offers viable alternatives for spoilage control and allows better targeting of formulation strategies.

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Thermal Inactivation of Staphylococcus aureus in broth

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OBJECTIVE

Spoilage and pathogenic bacteria can be present in foods even after cooking. It is restricted that the presences of potential pathogenic bacteria like *S.aureus* by the regulatory agencies in World wide. As a first step of our study it is aimed to determine the heat resistance of *S.aureus* at 55°C to 75°C.

METHODS

S.aureus ATCC 29213 was used at this study. To prepare the cultures one loop of colonies were transferred from the petri dish to 9.0 mL of Tryptic Soy Broth (TSB) and serial decimal dilutions were prepared by transferring 1.0 mL of the culture to another TSB and incubated for 24 h at 35°C. These cultures were not used in thermal heating studies. The inocula for use in heating studies were prepared by transferring 0.1 mL of each culture to 9 mL tubes of peptone water (PW) and incubated aerobically for 24 h at 35°C. These cultures were maintained by consecutive daily transfers in PW for 1 week. The population densities in each cell suspension were determined by surface spread plating appropriate dilutions, in duplicate, on Nutrient Agar (NA). The broth suspensions were heated at 55, 60, 61, 62, 63, 64, 65, 70 and 75°C using a time-temperature controlled water bath. During the heating process, samples (1 mL) were removed at predetermined time intervals. Samples were cooled immediately under cold tap water. Decimal serial dilutions were prepared in PW and appropriate dilutions surface-plated in duplicate on NA. All plates were incubated at 35°C for 24-48 h prior to counting colonies. For each replicate experiment, an average of each sampling point was used to determine the D-values (Juneja et al. 2001).

RESULTS

Both 55°C and 60°C for 4 minutes were insufficient degrees of the thermal death process. However, cultures were inactivated at 65, 70 and 75°C for 4 minutes. Apart from these data, cultures were survived at 61°C for 4 minutes, 62°C for 3 and 4 minutes, 63°C for 3 and 4 minutes and 64°C for 2 minutes. Based on the pooled data, the estimated 100% killing time was 4 minutes at 65°C in PW when the initial concentration of *S.aureus* was 10^7 cells/mL. The present data could be fitted into the following linear regression model:
y (log bacteria) = -1,753 x (time) + 7 (r^2=0,999)

This equation of viable counts corresponding to a D_{65} value of 0.57 minutes. When considering the earlier literature, thermal sensitivity of the most *S.aureus* strains inactivated at 54 to 60°C for 4-24 minutes. However, our findings demonstrated that below 65°C degrees were not appropriate for the purpose of completely killing of the bacteria. In addition, it is also well documented that above 60°C degree prevents enterotoxin production when storing/serving the foods (Karapinar et al. 1998).

CONCLUSIONS AND IMPACT OF THE STUDY

This research was undertaken to design a thermal process for manti production. It is a traditional Turkish dish consumed after cooked either in water or in oven. It comprises meat, wheat flour, onion and red pepper. A heat treatment was used prior to packaging. More recently, a work has performed for determining an optimum heating process from the view of the sensory point (Uzunlu 2012). Therefore, it is aimed to determine whether an optimum thermal inactivation process should be handled by predicting the results of

this study. The present study can be used to predict the time required at specified temperatures to obtain thermal inactivation rates such as 7 log reductions of *S.aureus*.

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Modelling the thermal inactivation of Verocytotoxin Escherichia coli serotypes in buffalo milk curd during stretching phase

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OBJECTIVE(S)

The inactivation of potentially contaminating zoonotic bacteria in buffalo mozzarella is ensured through surveillance at the production farms including a heat treatment of the curd in hot water. However, several studies carried out in Southern Italy indicated a high carriage rates of verocytotoxin-producing (VTEC) *Escherichia coli* in buffalo's herds (14.5-32.3%), and isolates of serotype O157:H7 have been detected (Galiero et al. 2005).

The objective of this study was to evaluate the thermal inactivation of verocytotoxin-producing *Escherichia coli* in buffalo curd. Thermal inactivation was described using first order inactivation models at different treatment temperatures and further validated with literature data.

METHODS(S)

The bacteria used in this study included a no-fermenting sorbitol strain; VTEC O157 (O157, Vt1+, VT2+, eae+, Hly+) isolated from buffalo milk, and a no-fermenting rhamnose VTEC O26 (O26, Vt1+, eae+).
Curd, approximately 200 g, was brought from a local artisanal cheese plant producing mozzarella. Challenge tests were carried out at different temperatures (68°, 70°, 73°, 77° and 80°C). For each test thirteen tubes (replicates) were put in a thermoblock (Thermostat Plus, Eppendorf, Germany). Treatment times were set at 2, 3, 5, 7 or 10 minutes and at the end of each period, a couple of tubes were immediately chilled in the water bath. Counts of viable VTEC were done with MPN-PCR method to confirm the presence of the targets VTEC (O157 or O26) using the serogroup specific rfbEO157 and wzxO26 primers designed by Perelle et al. (2004).

A log linear-tail model (Geeraerd et al., 2000) was fitted to observed data. Kinetic parameters, such as maximum inactivation rate (k_{max}, min^{-1}) and residual population (N_{res}, log MPN/g) were calculated for both strains. RMSE and R^2 were used as measures of the goodness-of-fit. Additionally, D-values (min) were estimated at each temperature as the inverse of k_{max} and the time needed to produce a 4D reduction was calculated (TT-4D).

Further, an ANOVA analysis was performed to evaluate significant differences between the strains and temperatures tested ($p<0.05$).

RESULTS

The initial VTEC counts were in the range between 7.2 and 7.7 mean log MPN/g for the curd samples inoculated with the strain of serogroup O157 and in the range between 7.1 and 8.7 for those inoculated with the strain of serogroup O26. Their numbers (mean MPN values) were reduced of more than 3 log in all samples after thermal treatments at temperature between 68°C and 80°C for 2 min but a slight lower reductions up to 2.0 log MPN/g have to be considered due to the uncertainty due to MPN counting method.

A good adjustment was obtained with the model used since values of R^2 were higher than 0.87. For *E. coli* O26, k_{max} increased from 3.94 to 4.50 min^{-1} at 68 and 80ºC, respectively. Higher resistance was observed for *E. coli* O157 at 68ºC, since k_{max} was 1.48 min^{-1}. Application of high temperatures (80ºC) during the stretching phase produced a residual population lower than 10 MPN/g for both strains at the end of the treatment. Significant differences were denoted ($p<0.05$) for N_{res} between the temperatures tested for both strains, which implied the presence of resistant subpopulations of *E. coli* at temperatures lower than 73ºC.

CONCLUSIONS AND IMPACT OF THE STUDY

The thermal inactivation models developed in this study provide the basis for the definition of alternative process criteria that, along with the assessment of Performance Objectives at the primary production (qualification of suppliers), allows the validation of Critical Control Points throughout the food chain.

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Thermal Tolerance of Some Microorganisms in Manti: Determination of D-values

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OBJECTIVE

Manti is a traditional Turkish dish consumed after cooked either in water or in oven. It comprises meat, wheat flour, onion and red pepper. After application of heat treatment generally in boiling water for 10-15 minutes, it is served with garlic yoghurt sauce, dried mint and sumac. Variable heat treatment temperature and time values were applied prior to packaging, depending on the final product. Therefore, it plays an important role for inactivating the initial load and sensory characteristics. It is aimed at this study to determine the thermal inactivation rates of natural initial microbial load of manti at 60°C for 4 minutes.

METHODS

Wheat flour, minced meat, dry onion, black pepper, salt and water were used as raw materials of mantı. Dough material were prepared by mixing wheat flour and water at a 2:1 ratio (w/v) to obtain 0,1925 mm average sheeted dough. The filling was prepared by mixing minced meat, kneaded dry onion, salt and black pepper. Filling material were subjected to the each square of dough material in an equal amount of an average of 4 measurements with 1,275 g. Product left then to the cooling process at 20°C for 30 minutes. The heat treatment was performed with an oven at a product center temperature of 60°C and treatment time of 4 minutes. These values were earlier determined by trying at various degrees by judging appearance and texture attributes of the product (Uzunlu 2012). Total aerobic mesophilic microorganisms (TAMB), *Lactobacillus* spp., yeast and *S.aureus* analyses were performed before and after the heat treatment by plate counting of viable colonies on the media, documented at the standard routine analyses methods.

RESULTS

Based on the obtained data, the estimated 100% killing time was 4 minutes at 60°C in manti when the initial concentration of 10^5, 10^4, 10^3 and 10^2 cells/g for TAMB, *Lactobacillus* spp., yeast and *S.aureus*, respectively. The present data could be fitted into the following linear regression models:

y (log bacteria) = -1,406 x (time) + 5,61 (r^2=0,998)
This equation of total aerobic mesophilic viable counts corresponding to a D_{60} value of 0.71 minutes.
y (log bacteria) = -1,187 x (time) + 4,75 (r^2=0,999)
This equation of *Lactobacillus* spp. viable counts corresponding to a D_{60} value of 0.84 minutes.
y (log bacteria) = - 0,944 x (time) +3,77 (r^2=0,999)
This equation of yeast counts corresponding to a D_{60} value of 1.06 minutes.
y (log bacteria) = - 0,64 x (time) + 2,56 (r^2=0,998)
This equation of *S.aureus* viable counts corresponding to a D_{60} value of 1.56 minutes.

CONCLUSIONS AND IMPACT OF THE STUDY

Cooking is still remains of eliminating pathogens from muscle foods and protecting consumers against foodborne disease. When considering the death reduction of microbial population is fitted to the first order kinetics, when a given temperature the reduction in the log numbers of viable counts occurs in a linear manner over time. Log-linear thermal death time model gives a good fit to the inactivation data only when

death occurs rapidly (Juneja et al. 2001). Results of our study complied with the literature. The success of shelf-stable manti production is belonging to the lowest microbial load prior to packaging. There after the other factors such as packaging methods and storage conditions might be take attention. It should be underlined that the good manufacturing conditions and product quality should be implemented during the processing. Temperature treatment should be applied above 60°C degree.

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Growth potential of Listeria monocytogenes in traditional Austrian cooked-cured meat products

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OBJECTIVES

European Union legislation sets limits for *Listeria monocytogenes* in ready-to-eat foods (EC, 2005), depending on whether or not foods favour the multiplication of this bacterium, which is to some extent related to water activity (a_w) and pH. A peculiar group of traditional Austrian meats incriminated in human foodborne listeriosis (Pichler et al., 2009) are products made from cured cooked comminuted meat with blood ("Blutwurst") or gelatin/aspic ("Presswurst") as binders; the level of heat treatment may vary from ca. 50 to >80°C. Other fillers may be added, differing between regions and manufacturers. The intrinsic (pH, NaCl or a_w) and extrinsic (atmosphere, temperature) factors can be measured quite easily, and based on these data, growth of the pathogen can be assessed via mathematical models and predicted results be validated by challenge tests. The aim of this study was (1) to define the range of intrinsic and extrinsic factors (extreme and average); (2) predict the response of *L. monocytogenes* to these factors; (3) to validate the predictions by challenge tests, in order to define under which conditions the afore mentioned foods can be considered as "safe".

METHODS

Jellied pork (n=10) and "Blutwurst" (n=6) obtained from 10 different producers in/around Vienna were analyzed for proximate composition and pH. *L. monocytogenes* growth was estimated using the Combase growth predictor. Shelf life studies were based on the respective EU guidance (EC, 2008). Samples were inoculated at a level of ca. 100 cfu/g with a mix of *L. monocytogenes* NCTC 11994 and four field strains isolated from local meat processing plants. Samples were vacuum packed individually and stored at 2, 4, and 8°C. Listeriae were enumerated at days 1, 3, 6 and 9 on ALOA medium.

RESULTS

For jellied pork and "Blutwurst", pH ranged from 4.9-6.3 and 6.5-7.1, respectively. Ranges for NaCl were 1.4-2.5%, or 1.3-1.9%. Given a shelf life of 9 days at +4°C, predicted changes (ComBase) in numbers of *L. monocytogenes* were 0.1-1.7 log for jellied pork assuming that listeriae were not cold-adapted, or ≥1 log when full adaptation was assumed (no lag phase). For "Blutwurst", growth potential was 1.7-1.8 log for non-adapted and >3 log for cold-adapted *L. monocytogenes*. The predictions suggested that the technologically available range of NaCl concentrations would have no effect on *Listeria* growth, whereas low pH achieved in certain jellied pork products would. Likewise, challenge tests conducted with cold-adapted *L. monocytogenes* on Blutwurst of "average" intrinsic properties showed potential to support growth. During 9 days, *Listeria* increased by 1.9, 3.1 and 2.9 log, at storage temperatures of 2, 4, 8°C. However, when non-adapted listeriae were inoculated, growth potential rarely exceeded 0.5 log. In contrast, Presswurst (pH 5.7; 1.3% NaCl) was not able to support growth of *L .monocytogenes* (<0.5 log) at all 3 different tested.

CONCLUSIONS AND IMPACT OF THE STUDY

This pilot study demonstrated the potential of *L. monocytogenes* to multiply on traditional Austrian meat products, under the condition of vacuum package at 4°C. In first experiments, the significance of the "history" of contaminant listeriae could be shown. Variation in salt content will barely affect growth of this bacterium, but adjustment of pH may significantly retard *Listeria* multiplication. Further studies will explore the potential of acidulants and other process factors in order to improve food safety.

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Growth model of Escherichia coli O157:H7 at various storage temperatures on kale treated by thermo-sonication combined with slightly acidic electrolyzed water

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OBJECTIVE(S)

The objectives of this study were to investigate the disinfection efficacy of thermo-sonication combined with slightly acidic electrolyzed water (US+SAcEW) for inactivating *Escherichia coli* O157:H7 on kale and to develop a model for describing the effect of storage temperature (4, 10, 15, 20, 25, 30 and 35ºC) on the growth of *E. coli* O157:H7 on fresh-cut kale treated with or without (control) thermo-sonication combined with slightly acidic electrolyzed water.

METHOD(S)

The two acid tolerance *E. coli* Ol57:H7 strain ATCC 43894 and ATCC 43895 were used for spot inoculation (10^8 CFU/mL) onto the surface of fresh-cut kale (*Brassica oleracea*). All samples were treated with SAcEW (pH of 5.5-6.0, ORP of 950–1020 mV, and ACC of 17-25 ppm) placed in an ultrasonic probe system (frequency of 40 kHz and acoustic energy density (AED) of 400 W/L) with sublethal heat of 40ºC for 3 minutes, and then washed with 200 mL neutralizing agent (0.85% NaCl containing 0.5% $Na_2S_2O_3$). Each 10 g samples without inoculation, untreated or treated samples inoculated with *E. coli* O157:H7 were incubated at 35ºC for 24 h. For shelf-life study, the untreated and treated samples were stored at various storage temperatures (4 - 35ºC). During storage, sampling was carried out at 2-day intervals for 4ºC, while only 3-h intervals for 30 and 35ºC. Generally, lower temperatures resulted in longer sampling intervals, while shorter intervals for higher temperatures. Each experiment was replicated three times. The growth curves were fitted with the modified Gompertz equation. The square root model and the natural logarithm model were established for the specific growth rate (SGR) and lag time (LT), respectively. Validation of the developed models based on the additional experimental data not used for the model development.

RESULTS

The bactericidal effects of the combination treatments against *E. coli* O157:H7 on kale were approximately 3.35 ± 0.08 log reductions. The modified Gompertz model was fitted well with these growth curves that provided a good statistical fit ($R^2 > 0.98$). SGR increased and LT declined with rising temperatures in all samples. Higher SGRs and lower LTs were observed significantly in treated samples compared to control. However, the number of bacteria on treated samples was always significantly lower than control; in other words, the treated kale can be stored for a longer time than control. Similar results have been published in the use of alkaline electrolyzed water and slightly acidic electrolyzed water as a sanitizer alone (Ding et al., 2009 and 2010). From the secondary models, the values of R^2 ($R^2 > 0.93$) and the values of the R^2_{Adj} ($R^2_{Adj} > 0.91$), indicated that the models could give good predictions. All B_f values were in the range of 0.99–1.04 and A_f values of 1.03–1.07, indicating a perfect concordance (Ross, 1996). In addition, The lower of standard error of predictions (%*SEP*) value obtained in this study proved the better performance of this predictive model.

CONCLUSIONS AND IMPACT OF THE STUDY

The combination treatments of thermo-sonication and SAcEW had strong bactericidal effects and the overall predictions had slight deviation with the observations for its acceptable ranges of bias and accuracy factors

as well as lower standard error of predictions, which indicated that the presented growth model can be used to assess the risk of *E. coli* O157:H7 infection on kale.

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Predicting outgrowth and inactivation of Clostridium perfringens in meat products during low temperature long time heat treatment

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OBJECTIVE

Sous-vide cooking and molecular gastronomy has started a wave of experimenting with Low Temperature Long Time (LTLT) heat treatments. Heat treatments, at temperatures as low as 50°C, have been suggested by celebrity chefs. LTLT treatments often take hours to reach to the final core temperature and *Cl. perfringens* is, therefore, of special interest as it may outgrow during the coming up time and cause food safety problems. This study was undertaken to set up a predictive tool to establish the outgrowth potential of *C. perfringens* in LTLT meat products as a function of the applied heating profile.

METHODS

Challenge tests were performed at two dynamic temperature profiles (fast LTLT, 2.6 h from 10 to 53°C, and slow LTLT, 3.8 h from 10 to 53°C) with three types of inoculums (spores, heat-active spores and vegetative cells) of *Cl. perfringens* 790-94 in two different types of meat pork (pH 5.6) and chicken (pH adjusted to 6.8). Challenge tests representing LTLT treatments of beef were collected from the literature. The obtained growth data were used for evaluation of three different growth models originally validated for prediction of growth during cooling (Le Marc et al. 2008, Juneja et al. 2011, Jaloustre et al. 2011). The data in inactivation phase were used for evaluation of three inactivation models generated by Foegeding and Busta (1980), van Asselt and Zwietering (2006) and Jaloustre et al. (2012). Finally, a new growth model, derived from the model structure of Le Marc et al. (2008), was developed from literature data (215 isothermal growth data), and was combined with a linear inactivation model developed from data at 53°C from this study for completely predicting fate of *Cl. perfringens* during LTLT treatment.

RESULTS

Very short lag times were observed in most of the challenge tests, especially in high pH chicken. By using the acceptable prediction zone method, performance of literature models was evaluated and none of the growth and inactivation models could successfully predict the growth or inactivation of *Cl. perfringens* for the LTLT conditions of our challenge tests. Therefore, a new growth model and a new inactivation model were developed and combined to predict the overall fate of *Cl. Perfringens* during LTLT profiles at 53°C. However, predicted lag time was still much longer than the observed lag time in our challenge tests, which caused a general underestimation of growth giving rise to overestimation of inactivation in particular for chicken. To obtain more precise predictions, an RLT of 2-3 was recommended for chicken, independent on type of inoculums, and for vegetative cells in pork. For spores and heated spores in pork the increase of *Cl. perfringens* during LTLT coming up time never exceeded 1 $\log_{10}$-unit.

CONCLUSIONS AND IMPACT OF THE STUDY

A model combining both growth as well as inactivation for prediction of fate of *Cl. perfringens* during the LTLT treatment was developed. The model is the first predictive model specifically designed for LTLT treatment of meats. Very short lag times were observed during LTLT treatments, which were not observed in isothermal or cooling conditions previously. The reason for short lag time during slowly increasing temperature conditions, and how to predict it, should be interesting for future studies.

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Evaluation of Antifungal Activity of Crude Extracts from Zizyphus spina Christi L. Desf. and Peganum harmala L. on Fungal Species Development

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OBJECTIVE(S)

The Algerian Sahara is very rich vegetable in cash likely to have therapeutic properties. This work studies the antifungal activity of medicinal plants extracts from the South-West of Algeria: *Zizyphus spina Christi L. Desf.* and *Peganum harmala L.*

METHODS(S)

Aerial part from each plant is used for extraction by three solvents: water, methanol and hexane. Antifungal activity of the different extracts was studied witch respect to seven fungal strains, on the growth of the mycelium, germination and the sporulation. For both tests, six extract quantities were used (100, 200, 400, 700, 1000 et 1500 µg/1 ml of medium PDA).

RESULTS

Among all solvents, methanol gave the best extraction yield for Peganum harmala L. (24, 02%) and water for Zizyphus spina Christi L. Desf. (25, 27%). The phytochemical screening [1] indicates that our plants are relatively rich in active consisting: flavonoids, saponins, sterols, terpenes, steroids and tannins.

The results of method direct contact on mycelial growth, shows that the three extracts of Peganum harmala L were more active against fungi with extracts of Zizyphus spina christi L. Desf. With a concentration of 1500 µg/1ml the most important effect for the 1st plant was observed by the methanol extract, whose, F. oxysporium, Alternaria, A. ochraceus and Cladosporium are most sensitive, with percentages of inhibitions respectively of 68,67%,71,15%, 80,39% and 95%. Whereas for the 2nd plant the extract most active was the hexanic extract, the most sensitive stocks are F. oxysporum and A. Niger with an inhibition of 80,95% and 50%.

In addition to the growth of the mycelium, the various extracts of two plants showed, in vitro, a antifungal activity at least important on the two other developmental stages, germination and the sporulation, of all fungi, is showed an inhibiting action of the spores which exceeds 50% of all fungi.

CONCLUSIONS AND IMPACT OF THE STUDY

The effect of plants extracts on growth fungi, *Peganum harmala L* extracts showed great efficacy than did *Zizyphus spina christi L.*

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Evaluation of cauliflower, broccoli, and okara agri-food by-products antimicrobial capability by mathematical modeling against Salmonella enterica serovar Typhimurium

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Large amounts of agri-food by-products are generated by food processing industry every year, with important economic and environmental associated problems. Nowadays, many studies are focused on recovery, recycling and upgrading these by-products for use them as operating supplies or as ingredients of new products formulation because due to they could be a valuable source of nutritional and antimicrobial compounds. To our knowledge, no previous studies deal with the mathematical modeling of microbial inactivation by means agri-food by-products intervention. Therefore, the aim of the present study is to evaluate mathematically the antimicrobial potential of cauliflower, broccoli, and okara by-products from primary production against one of the most concerned foodborne pathogens, *Salmonella enterica* serovar Typhimurium. The kinetic growth behavior of *S.* Typhimurium was described at 37 °C incubation, under exposure to the studied vegetable by-products added at 5% (w/v) in reference medium. All studied by-products, cauliflower, broccoli and okara, showed bactericidal effect against *S.* Typhimurium. The most promising results were achieved in the binomial cauliflower– *S.* Typhimurium, because the bacterial population was reduced by 3.11 $\log_{10}$ cycles after 10 h of incubation at 37 ^{o}C as a result of 5% (w/v) cauliflower addition. Based on this results, a deeper study was carried out for this combination, at different cauliflower concentrations (0, 0.5, 1, 5, 10, 15% (w/v)) and at temperatures in the range [5–37] °C. The greatest inactivation level (6.11 $\log_{10}$ cycles) was achieved under incubation at refrigeration temperature (5 °C) as a result of 15% (w/v) cauliflower addition. Results obtained demostrated that both temperature and cauliflower concentration significantly ($p \leq 0.05$) influenced the *S.* Typhimurium inactivation level. The Weibull mathematical model provided an accurate fit (RMSE [0.01–0.20] and *adjusted*-R^2 [0.95–0.98]) to experimental *S.* Typhimurium survival curves describing inactivation kinetics under the cauliflower antimicrobial effect. The Weibull kinetic parameters showed that the inactivation kinetics were convex in all cases (n>1) and the inactivation rates, which are directly related with b values, were greater at higher temperatures, which confirmed that higher temperature accelerated the microbial inactivation. According to the results obtained, cauliflower by-product can be considered as a potential material to be included as new additives or new ingredients in animal feed supplementation based on its antimicrobial properties. These assumptions should be scaled to in vivo systems, and could have remarkable consequences to agri-food industry reducing costs of raw materials and contributing to solve environmental problems.

Keywords: agri-food by-products, Salmonella typhimurium, natural antimicrobials, Weibull distribution function

Contribution to the study mycological and mycotoxicological some aromatic plants

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Many agricultural products are likely to contain toxigenic molds, including herbs that are often left after harvest on the ground in a dry environment, which is in most cases, the ideal conditions for mold growth and mycotoxin production. For this purpose, we contribute a mycological and mycotoxicological study for some herbs marketed in the south-western Algeria. All samples analyzed (cumin, caraway, chili, pepper and ras el hanoute) are contaminated with molds. We have identified various types of molds belonging to the flora of storage, other genre belonging to the field flora and intermediate flora. The test research of aflatoxin producing strains of differents isolates of *Aspergillus. Flavus* and *parasiticus* revealed that 50% of tested strains are producing AFB1 and AFG1 and 50% were considered non-productive.

The operating results for aflatoxins by TLC on our substrates were positive with the exception of samples Caraway has revealed negative.

Key words: moulds, mycotoxin, aflatoxin, aromatic plants

Modeling the combined effects on Listeria monocytogenes behavior in mono- and coculture experiment with Lactobacillus sakei and Staphylococcus carnosus.

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OBJECTIVE

In food processing it is necessary to yield alternatives that allow to increase the shelf life of perishables like meat products, because of susceptibility of these ready-to- eat foods to contamination by *Listeria monocytogenes* and other food pathogens, for this reason food industry has needed to ensure sanitary quality. For this reason models describing microbial growth is a helpful tool to design biopreservation methods and predict shelf life, especially through involved microbial interactions. The aim research was studied the addition of starter cultures, typical used for semi-ripening sausages like salami (*L. sakei/ S. carnosus*) on behavior of *L. monocytogenes* in vitro was evaluated by mathematical models. These models could be applied in control on fermentation and ripening meat product process.

METHODS

The strains used in this study were *Lactobacillus sakei* and *S. carnosus*, belonging to the starter culture T-SC-150 Bactoferm™ (CHR-Hansen), and *Listeria monocytogenes* belonging to culture collection of the Department of Engineering of La Sabana University. *L. sakei* and *S. carnosus* was isolated and identified by (GTG)5-PCR fingerprinting. The bacterial growth was analyzed in combined effects (ratio initial bacterial concentration between starter culture and pathogen, aerobic or anaerobic conditions, sodium nitrite addition as well as fermentation and ripening temperatures). Initial bacterial concentration of *L. sakei* and *S. carnosus* was approximately of 6 Log ufc/ml each one and *Listeria monocytogenes* was 3.5 Log ufc/ml and 1.5 ufc/ml. Experiments in monoculture were measured by optical density: aerobic and anaerobic conditions, with 200 ppm sodium nitrite and two cycles of temperatures, these temperature conditions were chosen from state of the art review (17±0.1°C from 0 to 48h and 21±0.1°C from 49 to 120 h), and a real industrial process (21±0.1°C from 0 to 48h and 17±0.1°C from 49 to 120h). In co-culture was measured by total viable count, on aerobic conditions, 200 ppm sodium nitrite addition and a temperature 21±0.1°C from 0 to 48h and 17±0.1°C from 49h to 14 days, because in these temperature conditions in monoculture specific rate growth was maximum for *L. sakei* and minimum for *Listeria monocytogenes*, data for *S. carnosus* wasn´t having meaningful difference ($p>0.01$).

RESULTS

Specific growth rate and log phase was obtained by the polynomial model was compared with Gompertz modified and Baranyi - Roberts models, the dataset was compared through variance analysis and orthogonal contrast ($p<0.01$). According to combined effects. The models describing the behavior between *L. sakei* and *S. carnosus* in a starter culture, and this application as induced inhibition through reducing growth specific rate and increasing stationary phase of the pathogen.

In the development of a suitable model structure of mono- and coculture experiments to describe the influence of the sodium nitrite addition, aerobic and anaerobic conditions as well as different temperatures from fermentation and ripening used in salami process. As a result, the model is able to accurately predict the growth, inhibition and subsequent inactivation of *L. monocytogenes* in a real salami process and possibility to control this process.

Keywords: *Lactobacillus sakei*, *Staphylococcus carnosus*, *Listeria monocytogenes*, predictive microbiology, microbial interaction, salami process.

Modeling the effect of variations in fermentation temperature on the reduction of three pathogens during the production of dry fermented sausages

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OBJECTIVE

In a former project at DMRI, a dynamic model for predicting reductions of *Salmonella*, verocytotoxigenic *E. coli* (VTEC) and *L. monocytogenes* during the production of fermented and matured sausages has been developed (the Conferm model; Gunvig et al, 2013 and Gunvig et al, 2012). The model includes the variables; pH as a function of time, water phase salt (%), weight reduction (%) and sodium nitrite. The fermentation and maturation temperatures were 24°C and 16°C, respectively.

In this study the objective was to extend the ConFerm model with fermentation temperature as a variable.

METHODS

Three serotypes of *Salmonella* and VTEC, respectively and five strains of *L. monocytogenes* were cultured overnight in Brain Heart Infusion at 37°C. The cultures were mixed in equal proportions to the final inoculation cocktail, containing *approx*. 5 x 10^8 cfu/ml of each genus.

A total of 27 different recipes were tested, having different combinations of water phase salt (WPS), sodium nitrite content, and pH-decline. In order to obtain the different rates of pH-decline, three starter cultures were selected (T-SPX Bactoferm, F-1 Bactoferm, and FSC 111 Bactoferm; Chr. Hansen, Hørsholm, Denmark). In all recipes, 3 % NaCl was added to the mince, and by varying the fat content to approx. 10, 25 and 40 % in the final product, three levels of WPS were obtained. Three levels of $NaNO_2$ were tested: 0, 100, and 200 ppm. The fermentation temperatures were 18°C, 24°C and 28°C, the drying temperature was 16°C, and the humidity (85 – 90 %) during maturation.

The 27 recipes were tested at each fermentation temperature between one to three times, in total 161experiments (44, 73, 44 experiments at 18°C, 24°C and 28°C, respectively).

The observed reductions of the three pathogens were modelled with the inputs: weight loss, pH and WPS as a function of time, together with added $NaNO_2$, pH_{48} (pH after 48 hours), fermentation temperature and fat content of the final sausage using partial least squares regression (PLS-R) and cross validation.

RESULTS

Using 2 of the 3 starter cultures (F-1, T-SPX), a large difference in pH_{48} was observed, whereas FSC111 gave only minor or no change in pH_{48}, when fermenting at 18°C, 24°C or 28°C. After fermentation, also the observed reductions varied, depending on the fermentation temperature used. But in the finished product (30-35% weigh loss) no difference in "observed reduction" due to different fermentation temperature was seen. The PLS modeling procedure showed that the inclusion of fermentation temperature in the models provided no significant contribution to the models ability to predict reductions of the three pathogens in the final products. Typical differences in the standard error of prediction between models with and without fermentation temperature were in the range 0 – 1.5% relative which is well within the experimental uncertainty for the pathogen enumerations.

CONCLUSIONS AND IMPACT OF THE STUDY

The results indicate, that introducing the fermentation temperature as a variable in the ConFerm model will not improve the model, as this is adequately described by the pH decline during fermentation. The final reductions of Salmonella, VTEC and *L. monocytogenes* after 25-35% weight loss, are unaffected by variations in fermentation temperatures in the range 18°C to 28°C.

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Effects of the physiological state of Listeria monocytogenes and high pressure processing on relative lag times during growth in chilled RTE cooked meat product

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OBJECTIVE(S)

The growth behavior of *L. monocytogenes* on chilled RTE sliced cooked meat products was quantified and modelled taking into account the effect of high pressure (HP) processing and the physiological states of pre-cultures resulting from cold-adaptation or freeze-stress prior to pressure treatment.

METHODS(S)

Two different stationary phase pre-cultures of *L. monocytogenes* CTC1034 were studied: one was adapted to refrigeration at 8ºC and the other was frozen at -80ºC. The *L. monocytogenes* pre-cultures were used to inoculate cooked ham (a lean product) and mortadella (a fatty product) with 10^7 and 10^4 cfu/g. Samples were vacuum packaged, pressurized (400 MPa, 5 min, 15ºC) and chill-stored at 4, 8 and 12ºC. *L. monocytogenes* was periodically enumerated on ALOA to allow estimation of lag phase (λ) and maximum specific growth rate (μ_{max}) by using log-transformed cfu/g and the Logistic model with delay. The effect of storage temperature on μ_{max} and λ was modelled using the Ratkowsky square root model i.e. $\mu_{max} = (b\ (T - T_{min}))^2$ and the relative lag time (*RLT*) concept with *RLT* defined as lag time divided by generation and $\lambda = RLT * \ln(2)/(b\ (T - T_{min}))^2$ [5].

RESULTS

The physiological state of pre-cultures and HP treatment had no effect on μ_{max} of *L. monocytogenes* when subsequently growing in the chilled meat products. In contrast, the concentration of *L. monocytogenes* (log N_0) after HP treatment was on average 3.2 log cfu/g lower for cold-adapted cells compared to freeze-stressed cells. Thus the physiological state of pre-cultures markedly influenced inactivation by the HP treatment. In addition, for freeze-stressed cells the HP treatment resulted in significant lag times for all three chill storage temperatures.

The one-step regression approach [1] was used to develop secondary models for μ_{max} and λ in cooked ham (CH) and mortadella (M). Thus, μ_{max}-models parameters were b = 0.023 and T_{min} = -1.40 for CH, and b = 0.024 and T_{min} = -1.37 for M. The *RLT* parameter was: 6.43 (CH) and 11.6 (M) for freeze-stressed cells and 0.00 (CH) and 2.27 (M) for cold-adapted cells. The fitted *RLT*-values clearly reflected the impact of the physiological state of the inoculums on the observed lag times and the applied modelling approach allowed an empirical characterization and quantification. Two secondary models were necessary to describe the different growth behavior of *L. monocytogenes* on lean (CH) and fatty (M) cooked meat products, supporting the need of a product-oriented approach to assess growth after high pressure processing.

Interestingly, when considering both HP inactivation and subsequent growth of *L. monocytogenes*, the time to achieve a concentration 100-fold higher than the one prior to pressure treatment was similar for the two studied physiological states of the inoculum. The developed models provided reasonable predictions for both μ_{max} and λ for another *L. monocytogenes* isolate when equally stressed and growing on pressurized cooked ham or mortadella [2-4].

CONCLUSIONS AND IMPACT OF THE STUDY

In food processing, *L. monocytogenes* cells that could contaminate cooked meat products are likely to have various physiological states and thus the prediction of their response may seem complicated. Importantly, the present study indicates that the combined effect of HP inactivation and subsequent growth of *L. monocytogenes* in cooked meat products during chilled storage could be predicted irrespectively of the initial physiological stage of the pathogen.

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Quantitative analysis of Stevia rebaudiana Bertoni antimicrobial activity to guarantee the microbiological safety of a multi-fruit-oat juice processed by high hydrostatic pressure

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OBJECTIVE(S)

Fruit juices have been recognized to be targets of spoilage by yeasts, molds and acid-tolerant bacteria (Fitzerald et al., 2004). Furthermore, it has been demonstrated that even though pathogens do not actively grow in fruit juices due to their low pH, they can survive and adapt to the acidic environments, increasing their tolerance to unfavourable growth conditions (Mazzotta, 2001).

Thermal processing and the use of artificial preservatives have been traditionally used to inhibit and/or control the growth of microorganisms in foodstuffs. However, taking into account consumer preferences for minimally processed foods, without chemical additives, nowadays it is promoted the application of non-thermal technologies and the use of natural preservatives in order to ensure safety without modifying the nutritional value and the sensorial characteristics of the product (Barba et al., 2012).

For this reason, the main goal of this work was to evaluate the antimicrobial activity of *Stevia rebaudiana* Bertoni (*Stevia*) against *Listeria monocytogenes* in a beverage containing orange, mango and papaya juices and oat-milk, after being subjected to high hydrostatic pressures (HHP). This assessment was carried out by means of mathematical models, which allow characterizing and predicting bacterial response as a function of storage conditions, keeping in mind that this is fundamental for the planning of a good hazard analysis and critical control point system (HACCP) at industrial level.

METHOD(S)

Inoculated samples, with and without *Stevia*, were subjected to HHP and the kinetic behavior of the surviving bacteria was characterized at 37 ºC by viable count on tryptic soy agar, with and without sodium chloride (5%) (Somolinos et al., 2008), to assess the inactivation and the sublethal injury generated after processing and during the subsequent incubation, being the starting inoculum size 1×10^7 cfu/mL.

The *Stevia* extract employed was a leaf infusion 2.5% (w/v), and the treatments selected were the followings: 300 MPa/3 minutes and 300 MPa/1 minute.

The assessment of the preservative potential of the combination HHP and *Stevia* was done comparing the bacterial counts pre- and post-treatment, with and without salt, in absence and presence of *Stevia*, and comparing the counts and inactivation curves obtained during the storage of the treated samples. Experimental data were fitted to the Weibull distribution function given its simplicity and sturdiness to describe inactivation kinetics (Mafart et al., 2002), based on the "*b*" parameter value, directly related with the inactivation rate and inversely related with the time needed for the first decimal reduction takes place.

RESULTS

Regardless of *Stevia* concentration, the higher the treatment time, the lower the number of intact cells because the ratio of the inactivated and/or damaged cells is higher. This also occurs if pressure and treatment time remain constant and *Stevia* is added to the beverage, so, this ingredient enhances the effectiveness of

any of the treatments considered.

In addition it is observed that after HHP, *Stevia* accelerates the inactivation of surviving cells; meaning that the "*b*" value associated to samples with *Stevia* is higher than the "*b*" value associated with controls, and that the survival curves last less if the infusion is added to the beverage.

CONCLUSIONS AND IMPACT OF THE STUDY

The results obtained shown that high pressure processing in presence of *Stevia* can be a good strategy to inactivate and damage *L. monocytogenes* cells in beverages similar to the one formulated and to accelerate the inactivation of survivor during the storage of the product at non-stressful temperature conditions.

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Modelling the effects of NaCl and KCl on the survival of Salmonella in broth

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OBJECTIVE

The increasing trend for low sodium products has led to a requirement for more knowledge and data on the effect of replacing NaCl by other water activity (a_w) depressors on the inactivation of vegetative pathogens. Traditionally in food microbiology, the concept of a minimum a_w for microbial growth has been used to develop predictive models based on the assumption that the effects observed are solely a function of a_w. However, in some instances there are specific solute effects by which the microbial response at a given a_w differs when different solutes are used to control the water activity in the system. The objective of this study was to evaluate the effects of NaCl, KCl and combinations of NaCl and KCl on the inactivation of *Salmonella* at ambient temperature and neutral pH conditions.

METHODS

Seventeen experimental conditions at selected combinations of NaCl (0-25% salt/(salt+water)) and KCl (0-20% salt/(salt+water)) were tested at 23°C. The corresponding a_w levels ranged from 0.763 to 0.925. One hundred ml aliquots of Tryptic Soya Broth (TSB) were used as the base medium for preparation of the compositions. NaCl and/or KCl were added to the TSB to achieve the required concentrations. The pH was adjusted to 7.2 with HCl or NaOH prior to filter sterilisation. *Salmonella* was inoculated at a level of ca. 7 $\log_{10}$ cfu/ml and enumerated at various time-points for up to 57 days. The model of Cerf (1977) which describes the inactivation of two populations (susceptible and resistant) was used as a primary model. The secondary model is based on the model of Pin *et al.* (2011). Two equations were used (one for the susceptible population and the other one for the resistant population) and the proportion of the resistant population was assumed to be identical for all the survival curves. Model validation was performed by comparison with ComBase data.

RESULTS

The inactivation curves showed biphasic trends, which suggest the presence of two different populations (sensitive and resistant to low a_w). As the salt levels increased, the water activity of the test composition reduced and more rapid inactivation was observed. For example, at 14% NaCl (a_w 0.90) it took 30 days to achieve a 4 $\log_{10}$ reduction in numbers of *Salmonella* whereas at 20% NaCl (a_w 0.832) and at 25% NaCl (a_w 0.763), it took 20 and 15 days respectively. Equivalent levels of KCl did not exert the same level of inactivation as NaCl. However, similar inactivation results were obtained from broths with similar a_w values but achieved by different combinations of NaCl and KCl. This suggests that a_w (also predicted using molarity of humectants) could be used as the only explanatory variable in the model. The developed model describes accurately the observed kinetics (R^2_{adj}=0.97). The model exhibits either reliable or fail-safe predictions when compared to ComBase data.

CONCLUSIONS AND IMPACT OF THE STUDY

The study shows that by replacing some of the NaCl with KCl, similar *Salmonella* inactivation results could still be obtained, provided an equivalent a_w was achieved. The model developed may be useful in supporting the safe design of products with lower NaCl concentrations and ensuring rapid inactivation of *Salmonella*.

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Modelling the growth/no growth interface of Clostridium sporogenes spores in broth as a function of salt, nisin and potassium sorbate concentrations at two pHs

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OBJECTIVE

This study relates to hurdle technology, where several mild preservative factors are applied in combination, enabling minimal processing while providing assurance of food safety and quality[1]. To be successful, this technology requires the relative proportions of the individual factors to be carefully selected and tested. The aim of this study was to investigate the combined effects of the preservatives salt (sodium chloride), potassium sorbate and nisin on the survival of *Clostridium sporogenes* (*Cl. sporogenes*) and develop a model to predict the growth probability as a function of preservative concentrations. *Cl. sporogenes* can be used to represent *Clostridium botulinum* in studies aimed at controlling the growth of the latter[2].

METHODS

The effects of different levels of salt (0-4%), potassium sorbate (0-4%) and nisin (0-250 ppm) on the probability of growth of a cocktail of 5 strains of *Cl. sporogenes* (10^6 CFU ml^{-1} inoculum size) were evaluated in thioglycolate broth at two pHs (5.5 and 7) and 37ºC under anaerobic conditions over 8 weeks according to a full factorial experimental design (200 treatments). Ten replicates were prepared for each treatment combination. A cocktail was used to inoculate the samples in order to cover possible variability between strains. Therefore, the developed models can be considered safer than if developed with a single strain[3]. Each combination was observed for growth at 0, 1, 2, 4, 8 weeks by measuring the optical density at 595 nm using an automated micro-plate reader. The result was expressed as a probability value ranging from 0 (none of the replicates showed growth) to 1 (all ten of the replicates showed growth). The collected probability data were analysed using logistic regression to define the growth/no growth interfaces[1,5].

RESULTS

Analysis of the results showed that the main effects of salt, potassium sorbate and nisin on the probability of growth were significant ($p < 0.05$) at each of the two pHs (5.5 and 7). Also, the nisin-potassium sorbate and salt-nisin interactions were significant ($p < 0.05$) at both pHs. Quadratic effects of nisin and salt were significant at pH 5.5, while quadratic effects of nisin and potassium sorbate were significant at pH 7 ($p < 0.05$). The results of this study showed that lowering the pH from 7 to 5.5 decreased dramatically the levels of preservatives resulting in a given probability of growth.

CONCLUSIONS AND IMPACT OF THE STUDY

It can be concluded that the effects of salt, potassium sorbate and nisin on the probability of growth are additive. The inhibitory effect of potassium sorbate and nisin are strongly pH dependent and is greater at lower pH. A given probability of growth of *Cl. sporogenes* in broth can be achieved from the derived logistic regression models by appropriately manipulation of the levels of the preservatives at both pHs.

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Modelling the lag time of Bacillus cereus spores as a function of thermal heat-treatment intensity, product formulation (pH and a_w) and chilled storage conditions.

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OBJECTIVE(S)

Refrigerated Processed Foods of Extended Durability (REPFEDs) are popular, following consumer demand for chilled food products with long shelf lives and high organoleptic quality. For REPFEDs, it is generally agreed that a temperature of 90°C for 10 min will deliver a 6D inactivation of non-proteolytic *Clostridium botulinum*.

However, spores of psychrotrophic *Bacillus cereus* might survive such a pasteurization regime and germinate, after which the vegetative cells can grow up to a hazardous level. Consequently, the effect of heat treatment and product formulation on the subsequent lag time of surviving spores has a significant impact on the safety during shelf life.

The objective of this study was to develop a mathematical model to assess the effect of the factors related to the production process (heat treatment), the product formulation (pH and water activity (a_w)) and the distribution supply-chain (chilled storage temperature) on the lag time of heat-treated *B. cereus* spores.

METHODS(S)

A set of 434 growth/no-growth data, collected on two *B. cereus* strains by turbidimetry method (*1*), was re-interpreted as lag time values. The spore inoculum was deliberately high (10^4-10^5) to have a detection time close to the spore lag time. In the growth area, data were interpreted as observed values. In the no-growth area data were analysed as censored values (no growth within 60 days).

The factors of variation were heat-treatment intensity (85°C, 87°C and 90°C in a time range of 1 to 38 min), storage temperature (8-30°C), pH (5.2 - 6.4) and a_w (0.973-0.995).

A gamma-type model was developed, it had a conditional effect of heat-treatment in function of pH, i.e. the heat-treatment extended the lag time if and only if the pH was lower than its optimal. The model included the two strains within a unique structure, i.e. the residual error was considered to be constant whatever the strain. The model was solved by Bayesian inference with informative prior distributions, provided by literature or by Experts.

RESULTS

The data showed that a combination of stressful conditions significantly extended the lag time. For example, a combination of heat treatment at 85°C for 27 min, pH 5.6, a_w 0.987 and storage temperature 8°C led to a lag time longer than 60 days. The model was in full agreement with the observations. Likewise, at 10°C, after a pasteurisation at 90°C for 10 min and with a pH of 5.8, the lag time was predicted to be 11 days in a

product at high a_w (0.99) but longer than 50 days if the a_w dropped to 0.97.

The heat treatment as sole intervention was insufficient to extend the lag time more than a few days. Indeed, after a heat-treatment at 90°C for 10 min, required to control non-proteolytic *C. botulinum*, in a product at pH 6.2, a_w 0.99, kept at 10°C, the lag time was predicted to be no longer than 5 days.

Based on the model, various conditions of pH and a_w enabling an extension of the lag time up to 30 days, after a heat treatment of 90°C for 10 min, were calculated. For a product kept at 10°C, these combinations were for instance pH 6.0 - a_w 0.97, pH 5.7 - a_w 0.98 or pH 5.5 - a_w 0.99.

CONCLUSIONS AND IMPACT OF THE STUDY

An original gamma-type model describing the lag time of *B. cereus* heat-treated spores was developed. The factors were pH, a_w, heat treatment (temperature and time) and subsequent storage temperature. The model is a useful tool for REPFED producers to guarantee the safety of their products towards psychrotrophic *B. cereus*.

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Occurrence of toxigenic fungi in cereals and dried fruits commercialized in Algeria

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INTRODUCTION

Aspergillus species are the most important toxigenic fungi naturally occurring in warmer climates, both in field crops and stored foods. Cereals and dried fruits are among the commodities with the highest risk of aflatoxins (AFs) and ochratoxin A (OTA) contamination. The aim of this study was to investigate the presence of toxigenic fungi, aflatoxins (AFs) and ochratoxin A (OTA) from local wheat, rice and dried fruits from Algeria.

MATERIAL AND METHODS

A total of 268 samples (108 wheat, 110 dried fruits, 50 rice) were analyzed. The wheat samples were collected during pre-harvest, storage in silos and after processing. Dried fruits and rice samples from worldwide origin were purchased from different markets in Algeria. Dilution method and direct plating were used for isolation in DRBC medium (King et al., 1979). Malt Extract Agar (MEA) and Czapek-Dox agar (CZ) medium were used for morphological characterization. A preliminary screen for aflatoxin production was performed on CAM (Fente et al., 2001). Aflatoxins and OTA were analyzed using HPLC.

RESULTS

The results revealed that *Aspergillus* species are the most frequent in these commodities. *Aspergillus* section *Flavi* and *Nigri* being the predominant aspergilla. Among isolates of *Aspergillus* section *Flavi* examined, 45% produced high levels of AFs revealed in CAM and TLC. The most aflatoxigenic strains (> 90%) were identified as *A. flavus*. The most ochratoxigenic fungi isolated were *A.ochraceus, A. alliaceus* and *A. niger*. Aflatoxin B1 (AFB1) was detected by HPLC in 56.6% of the wheat samples with contamination levels ranging from 0.13 to 37.42 µg/kg, and in 90% of the dried fruits with contamination levels ranging from 0.16 to 25.82 µg/kg. Ochratoxin A was detected in 12 (40%) of the wheat samples at levels ranging from 0.21 to 41.55 µg/kg.

SIGNIFICANCE

Our results showed high contamination of cereal and dried fruits by aflatoxigenic and ochratoxigenic strains. Levels of AFs and OTA in these commodities seem to pose a risk to public health.

Keywords: Fungi, Aflatoxins, ochratoxin A, cereals, dried fruits Algeria

Quantifying the impact of strain, biological, and experimental variability on the growth kinetics of Listeria monocytogenes as function of pH

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OBJECTIVES

Quantitative modelling can be applied in product development and in food safety management systems. Deterministic models provide single estimates of kinetics, which often deviate from the real behaviour of microorganisms in question. The differences between the observed behaviour and predictions can result from experimental errors in the observed behaviour, biological variability of a strain, the variability between strains, , and product specific effects. Quantitative knowledge on these sources of variability allows integrating this in models and will result in a more realistic estimation of microbial kinetics behaviour and its variability. Therefore, in this project the impact of experimental, biological, and strain variability on the growth kinetics of *Listeria monocytogenes* as function of pH was quantified and compared to prioritize their importance.

METHODS

Twenty strains of *Listeria monocytogenes,* which cover a wide range of origins, were used in this study. The strains were grown in pH-adjusted medium (pH 7.3-4.2) and the two-fold-dilution method (1) was used to estimate the maximum specific growth rate (μ_{max}) as function of pH. Each experiment was conducted in duplicate to quantify the experimental variability and repeated 3 times on different days to determine the biological variability. Eleven secondary pH models, including one new proposed model, were used to describe the μ_{max} as function of pH for each strain and their performances were compared using four criteria: 1) having low mean square error, 2) having parameters with biological meaning, 3) having low number of parameters, and 4) giving realistic parameter estimates. The best model meeting those criteria was used further to estimate the pH_{min} of *Listeria monocytogenes,* and to quantify the strain variability in pH_{min} and μ_{opt}.

RESULTS

The strain variability was significantly higher than the biological and experimental variability, especially near the growth boundary (MSE strain>MSE biological>MSE experimental). From the eleven models that were able to fit μ_{max} as function of pH, the new proposed model was selected as the best model since it met all criteria. This allowed us to estimate the pH_{min}, μ_{opt}, and $pH_{1/2}$ that is the pH at which the μ_{max} is half of μ_{opt}. The degree of strain variation, expressed by the standard deviation of each parameter, was used to calculate the (95%) prediction intervals which were estimated as pH_{min} 4.54±0.17, μ_{opt} 1.00±0.09 h^{1}, and $pH_{1/2}$ 4.94±0.10.

CONCLUSIONS AND IMPACT OF THE STUDY

This study showed that variation between strains is larger than variation caused by biological and experimental error, and underlined the importance to quantify strain variability. The inclusion of strain variability and variability in growth limits into a generic model, such as the gamma model, can result in a better estimation of microbial growth kinetics including its variability.

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Microbiological hazard identification and exposure assessment of poultry sausages submitted to various high pressure processes and potassium lactate concentrations

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OBJECTIVES

Microbiological hazard identification and exposure assessment have been performed on poultry sausages treated by a promising food process technology used to control hazards in foods, *i.e.* high pressure processing. Poultry is indeed the most consumed meat in the world while poultry products are highly perishable. This study aimed at evaluating the potential use of high pressure technology in counterbalancing reduction of chemical preservatives, in the trend to potentially move towards clean label food products.

METHODS

First, a quantitative hazard analysis was carried out based on internal and external data. Internal data included microbial analyses (level and frequency) of raw materials, production line characteristics and product formulation. Besides, search from external sources (literature, food safety agency report, ComBase…) provided epidemiological data, hazard characterization (potential adverse effect) and microbial growth ability under various process and formulation conditions.

Next, the effect of high pressure treatment (level and duration) and potassium lactate concentration on inactivation of the identified microbial hazards were studied using a full factorial design. Sausages containing 0% or 1.8% potassium lactate (w/w), previously held at 4°C during storage, were inserted into the pressurizing chamber filled with water at 15±1°C and exposed to pressure of 200, 350 and 500 MPa, for 2, 8 and 14 minutes. Data derived from plate counts were analyzed using predictive microbiology tools.

RESULTS

Microbiological hazards were ranked in a semi-quantitative scale, illustrated into a 2D-chart with exposure levels at the end of product shelf-life on one axis, and hazard characterization on the other axis.

Microbial inactivation by high pressure was modeled using log-linear and Weibull functions. A secondary polynomial model was then used to assess the effects of high pressure and potassium lactate on the inactivation rate (either *D*-value or value). To determine the concentration of microbial hazards in the product at the end of the shelf-life, in case of i) their presence in raw materials and ii) their survival to the high pressure treatment, their potential growth in products after factory-release was quantified. Data and predictive models available in literature and Combase were used in this respect.

Finally, all the steps were integrated to estimate the level and frequency of contamination at the end of the product shelf-life.

CONCLUSIONS AND IMPACT OF THE STUDY

From the ranking of hazard exposure, *Listeria monocytogenes* and *Salmonella* were found to be the two main hazards of concern.

From the predictive model, inactivation of *L. monocytogenes* and *Salmonella* was shown to be significantly dependent on the pressure level (*e.g.* 3-log inactivation obtained when 500 MPa for 8 min was applied), while the potassium lactate concentration impact on the inactivation rate was negligible. Whatever the scenario (high pressure and lactate treatment combination level), the end-product contamination was estimated to be low and acceptable since the product is cooked by the consumer afterwards.

Constitution of a set of Listeria monocytogenes strains in order to perform shelf-life studies

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OBJECTIVE

The European Commission Regulation (EC) No 2073/2005 notified that *Listeria monocytogenes* mustn't exceed the limit of 100 cfu/g in ready-to-eat foods (RTE foods) throughout their shelf-life. Currently, the "Technical Guidance Document on shelf-life studies for *L. monocytogenes* in ready-to-eat foods" published by the European Union Reference Laboratory for *Listeria monocytogenes* (Beaufort *et al*., 2008) proposes a microbiological procedure for determining the fate of *L. monocytogenes* in RTE foods, by using challenge tests. These studies quoted in EC Regulation can be used by food business operators to be in compliance with the Regulation.

EURL Technical Guidance Document recommends using different strains to perform challenge tests to take into account the growth variability between strains.

Our study aims to explore intraspecific variability and to build a set of strains from various origins (isolated from meat, dairy, fish …) and with various genoserotypes (IV, II) and that have faster growth rate than other strains in harsh conditions (low pH, a_w and temperature).

METHOD

The constitution of a set of efficient strains was carried out in two steps. First, about 60 strains were selected from literature and from our laboratory for their specific growth abilities (set #A). Growth rates for these strains were characterised by spectrophotometry (Bioscreen) at low pH, low a_w and low temperature in broth medium. Method of the Times To Detection using multiple initial inocula was chosen to determine growth rate (Cuppers *et al.*, 1993). Inter-experimental variability was tackled for a pertinent inter-strains comparison.

Then within strains of set #A, one strain was selected for each growth conditions, genoserotype and origin based on its growth rate value to constitute a set of 16 efficient strains (#B). The strains of set #B were compared to randomly chosen strains (set #C) in broth medium for the three harsh conditions to confirm their abilities.

Finally growth rates of two strains (the fastest and the slowest of set #B) were obtained in two food matrices to check if differences observed in broth medium are still valid in foods.

RESULTS

According to growth rate values for strains of set #A, we showed that the origin and the genoserotype have a significant impact on growth rate. Strains originated from meat products presented higher growth rate at low pH, strains originated from dairy products are better at low temperature and seafood strains of Genoserotype IV are the fastest at low water activity.

No strains of set #C presented higher growth rate than strains of set #B.
Challenge tests carried out in the two foods corroborated the results obtained in broth.

CONCLUSION

The results of this project have allowed to explore the *L. monocytogenes* strains variability regarding the growth characteristics. *L. monocytogenes* strains of set #B could be provided to laboratories performing challenge tests.

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Response surface model for prediction of time to Staphylococcal enterotoxin A (SEA) detection in chicken breast broth as a function of temperature, pH and salt (NaCl) concentration

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OBJECTIVE(S)

This research aims to model time to toxin detection (TTD) as a function of abuse temperature, pH and salt concentration in chicken breast broth (normal pH 6.0), using a polynomial response surface model.

METHODS(S)

Inoculum: *Staphylococcus aureus* ATCC 13565 (SEA producer) was grown at 37°C/18h in BHI broth culture medium. Cells suspension was standardized at 10^8CFU/mL (Densimat, bioMérieux) and diluted up to 10^4CFU/mL.

Experimental design: Central composite, non-factorial design was applied using Statistica 7.0 (Statsoft, Tulsa, USA). The variables were temperature (16, 20, 30, 40 and 44°C), pH (5.3, 5.5, 6.0, 6.5 and 6.7) and salt concentration (0.8, 1.0, 1.5, 2.0 and 2.2%).

Meat preparation: Chicken breast meat was purchased from a local market, minced, distributed equally (25g) for each flask and sterilized at 121°C/15min. Seventeen different combinations of temperature, pH (adjusted after sterilization with 1N NaOH or 1N Tartaric Acid using pHmeter mPA210 MS TECNOPON) and NaCl concentration were obtained from experimental design. Each combination was prepared and inoculated with an initial population of 10^4CFU/g (final population ~ 10^3CFU/g).

Data collection and curve fitting: Each 8 hours of interval, a flask was removed from the incubator and an extraction protocol for enterotoxin detection was performed using VIDAS Staph Enterotoxin II (bioMérieux). Enterotoxin production was evaluated until 48h, since this is the product shelf-life. TTD was used as a response of secondary model. A polynomial response surface model was developed according to McKellar & Lu (2004). Simplified model was generated by backward stepwise regression, the non-significant effects ($p>0.05$) being excluded without damaging the model hierarchy, using mean of duplicate experiments. Mathematical verification of models was performed: bias and accuracy factors (Ross, 1996), R^2 and F_{cal} / F_{tab} relation.

RESULTS

Observed responses and response surface models

A total of 17 assays were conducted, and 13 (76.47%) were positive for toxin production. For 16°C and 20°C (pH 5.5 and NaCl concentration 1%) toxin was not detected in 48h (product shelf-life). The minimum TTD was 8h, observed for 40°C (pH 5.5/6.5 and NaCl concentration 2/1%) and 44°C (pH 6.0 and salt 1.5%). The maximum TTD was 32h at 20°C, pH 5.5 and NaCl 2%. For modeling, temperature (linear and quadratic) and salt concentration were significant ($p<0.05$). Obtained model gave a good prediction of the TTD (R^2=0.9802). The response surface model was established by stepwise regression as follow:

$$TTD = 16.2387 - 14.7292T + 6.3411T^2 - 2.0040S + 3TS \quad \text{(Eq.1)}$$

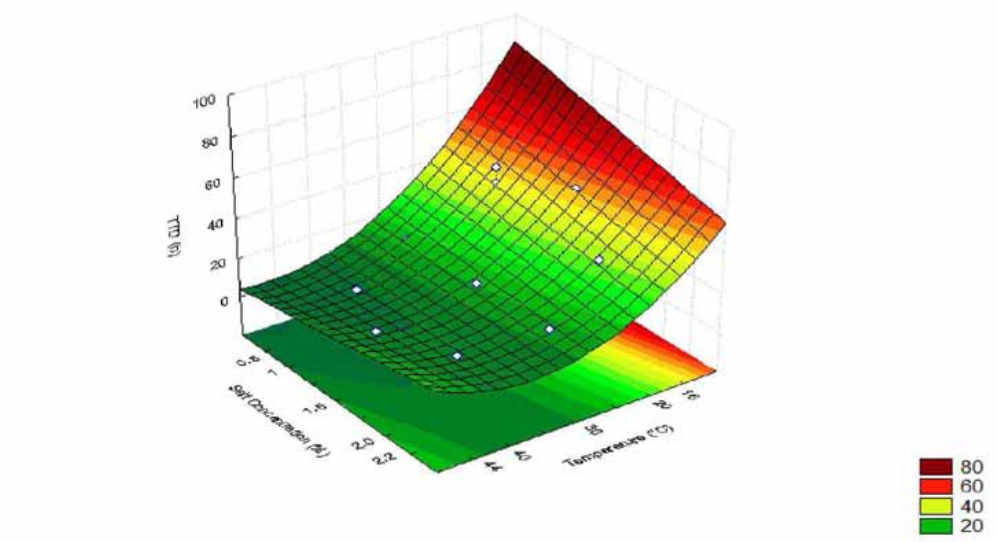

Figure 1. Response surface for TTD (h) as a function of temperature and salt concentration

Mathematical evaluation

Model mathematical parameters were good: R2 0.9802, bias factor 0.996, accuracy 1.075, Fcalc/Ftab 45.6. In comparison with previous researches, value of bias and accuracy factor of this evaluation was much lower and better (Dong et al., 2007). Figure 2 shows the correlation between predictedxobserved values, demonstrating that the model is not biased.

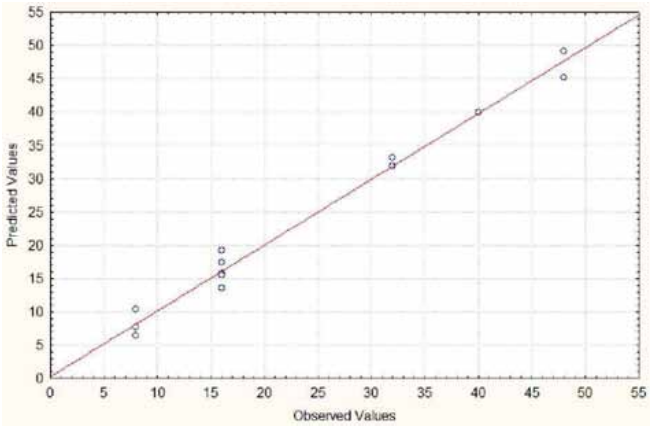

Figure 2. Plot of the observed and predicted values for secondary model.

CONCLUSION(S)

In summary, obtained model was able to predict the TTD with precision and can be applied in practice for predicting the TTD as a function of changes in salt concentration or temperature.

IMPACT

This model can be used by food microbiologists in order to prevent outbreaks caused by food intoxication. Knowledge of this work can also help people involved in food service.

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Development of a novel time-temperature indicator using the Maillard reaction for detecting Listeria monocytogenes growth during chilled storage

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OBJECTIVES

Listeria monocytogenes has been recognized as a critical pathogenic bacterium widespread in various foods and food-processing environments. In particular, chilled ready-to-eat (RTE) food is often associated with outbreaks of listeriosis because *L. monocytogenes* can grow in refrigerated foods. The temperature control and monitoring of RTE foods play key roles in the prevention of listeriosis outbreaks. Ideally, *L. monocytogenes* growth in chilled RTE foods should be identified before these foods are consumed. Time-temperature indicators or integrators (TTIs) are cost-effective and user-friendly devices to monitor, record, and translate the overall effect of temperature history on food quality and/or microbial growth in the chilled food chain. Although there have been numerous reports regarding the development of TTIs, none of these studies has focused on detecting and reporting the growth of *L. monocytogenes*. In addition, the majority of the previously developed TTIs have been based on the principles of molecular diffusion, polymerization reactions, enzymatic changes, and microbial changes. In the present study, we aimed to develop a novel TTI based on the Maillard reaction for monitoring *L. monocytogenes* growth.

METHODS

The TTI developed in the present study was based on the Maillard reaction. Aqueous solutions of D-xylose (4.0 M) and glycine (2.0 M) with K_2HPO_4 (1.0 M) were separately placed into a small plastic bag that was divided into two portions (0.3 ml each) using weakened heat-sealing. To begin TTI recording, the small plastic bag was crushed manually to break the partition and mix the two solutions. The solution colour then changes from colourless (clear) to pale blue, dark blue, and eventually dark brown over time (Hayase et al., 1999). The changes in the colour of the solutions were measured using a colorimeter (DR-200b; Minolta, Tokyo, Japan) and represented as L^*, a^*, and b^* values. The change in colour between the initial time point and an arbitrary duration was evaluated as ΔE. The changes in ΔE over time were examined at 4 to 20 °C and fitted with a logistic function. The changes in the ΔE of the TTI were simulated based on the parameters estimated from the iso-thermal experiments. *L. monocytogenes* growth was examined in brain heart infusion (BHI) broth at the same temperatures used in the TTI experiments. The *L. monocytogenes* growth was modelled based on the Baranyi model to simulate its growth under various temperatures and to correlate this growth with the changes in the colour of the TTI.

RESULTS

The changes in the ΔE of the TTI at each temperature were successfully described by a logistic function. The estimated parameters thus reflected the changes in ΔE of the TTI with an arbitrary temperature history. The minimum ΔE that could be recognized visually was approximately $\Delta E = 8$. Such changes in the ΔE of the TTI corresponded to an increase of nearly 1.0 $\log_{10}$ cycle in *L. monocytogenes* grown in BHI at the arbitrary temperatures examined. Thus, the measurement of the ΔE of the TTI through simple visual inspection enabled the identification of a risk of *L. monocytogenes* growth in products with an arbitrary temperature history (ranging from 4~20°C).

CONCLUSIONS AND IMPACT OF THE STUDY

The developed TTI based on the Maillard reaction can be used to detect the risk of *L. monocytogenes* growth in food products with an arbitrary temperature history. Because the rate of the Maillard reaction can be controlled by the concentration of the reactants (in this study, xylose and glycine), the flexible design of TTIs corresponding to specific risk factors (microorganisms) and/or food products should be achievable.

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A predictive model of the growth of Staphylococcus aureus and starter culture during milk fermentation in raw and pasteurized milk

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OBJECTIVE(S)

The objective of this project was to develop a predictive mathematical model of microbial dynamics and biochemical changes during the milk fermentation process. A secondary objective was to determine the effect of milk pasteurization and starter culture on the dynamics of *S. aureus*, lactic acid and pH in milk during the fermentation process. A third objective was to identify the relative effects of type of milk, pH and lactic acid on the growth of *Lactococcus lactis* and *S. aureus* in milk.

METHODS(S)

Differential equations were developed to describe the dynamics in *S. aureus*, *L. lactis*, pH and lactic acid during milk fermentation. The model was based on previous models of the interaction between *Yersinia enterocolitica* and *Lactobacillus sakei* on modified Brain Heart Infusion medium via lactic acid and pH (Vereecken et al., 2002, 2003; Jansen et al., 2006).

The experimental procedures, culture conditions and monitoring procedures are described in Withers et al., (2012). Briefly, microbial growth measurements of co-cultures of *L. lactis* and *S. aureus* were made in raw and matched pasteurized milk at 30°C. Experiments were carried out using two starter cultures A and B. Experiments were performed with high and low inoculum of *S. aureus*. The experiments were repeated as two trials. Microbial counts, pH and total lactic acid values were recorded hourly for the duration of the experiment (12 hours).

The system of differential equations was solved using Matlab software and the model was fit to the measurements using nonlinear regression for multiresponse data (Bates & Watts, 2007). Markov chain Monte Carlo (MCMC) was used for model parameter estimation (Gilks et al., 1996). The model parameters were assumed to be influenced by a sum of effects due to differences in growth medium, bacteria, starter, dose, milk and trial. Statistical analyses were performed in R.

RESULTS

The model can be used to predict the growth of pathogens and starter cultures in pasteurized/raw milk along with associated changes in the concentration of lactic acid and pH.

Applying the model to the observed dynamics of *S. aureus* and *L. lactis* in raw and pasteurized milk we found that milk medium had a significant effect on the *L. lactis* lag time (P=0.004), with a longer lag time in raw milk, possibly due to interaction with other resident bacteria in the raw milk. The pH was lower in the pasteurized milk and this was associated with higher lactic acid concentration and *L. lactis* numbers. However, there was no significant effect of milk medium or starter on *S. aureus* growth, lag time or sensitivity to pH.

We also found a significant difference between growth rate and lag time of the two starter cultures (P=0.00025). Starter A grew faster and resulted in more lactic acid production and lower pH.

CONCLUSIONS AND IMPACT OF THE STUDY

The effects of milk medium on pH are due to the delayed growth/start of biological activity of the starters. pH did not appear to affect the growth of *S. aureus* indicating that the growth of *S. aureus* cannot be halted via typical pH changes during milk fermentation. This is consistent with the findings of Rosengren et al., (2013) who concluded that *S. aureus* is able to grow and produce enterotoxin A within the pH and undissociated lactic acid ranges typical for a large part of the fermentation process during cheese making.

The model can used to describe the likely fate of pathogens during raw milk fermentation and assist in the design of safe food production practices.

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Interaction between temperature, pH and dissolved carbon dioxide on Listeria monocytogenes growth

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OBJECTIVE(S)

When CO_2 is introduced into the food package, it is partly dissolved in the food product. That is why the inhibition of microorganisms in modified atmospheres has to be estimated from the concentration of dissolved CO_2 in the aqueous phase (Devlieghere et al., 1998).

Carbon dioxide solubility is widely modified by temperature, and carbonic acid dissociation is influenced by pH. However, in the most of publications relating the CO_2 effect on bacterial growth, only the initial conditions of pH and atmospheric CO_2 concentration were adjusted. The acidification and the atmospheric concentration modifications caused by the CO_2 dissolution are not controlled. The aim of this work is (i) to study the bacterial growth in static condition of temperature, pH and atmospheric carbon dioxide thanks to adapted experimental protocols, and (ii) to model the impact of these 3 environmental factors and their interactions on *Listeria monocytogenes* growth.

METHODS(S)

The culture medium is the BHI (Brain Heart Infusion, Difco) supplemented with 0.2% of glucose, 0.3% of yeast extract. Caps employed to close flasks, are a connection system permitting a gas circulation, to maintain a constant atmospheric composition, despite of the CO_2 dissolution. During all the bacterial growth acquisition, a gas flow assures a constant concentration in head space of the culture media.

To adjust the pH at studied levels (pH 5.5 and pH 7), NaOH 5M is added before placing the media under gas flow. The NaOH volume to add is calculated taking into account the solubility (vs temperature) and the constant dissociation (Ka) of the carbonic acid. So, flasks are placed in temperature incubator under gas flow until stabilization of the pH. Medium is also balanced with head space, pH is verified, and medium is inoculated with *Listeria monocytogenes* ADQP105. At different time, a sample is plated to determinate the population size. Growth rate is estimated by fitting a primary growth model.

RESULTS

Growth rate is acquired following a factorial experimental design between temperature (8, 12 and 37°C), pH (5.5 and 7) and CO_2 concentration in head space (0, 20, 40, 60, 80 and 100%). Results show no significant effect of CO_2 concentration at 37°C. An inhibitory effect of CO_2 appears only at low temperature (8 and 12°C). The low solubility of carbon dioxide at warm temperature explains the no significant effect at 37°C. Regardless of the temperature, the CO_2 inhibition is not significantly different between pH 5.5 and pH 7. The pH does not affect the solubility, but only the dissociation of the carbonic acid. The pH only modifies the HCO_3^- concentration, while the CO_2 concentration is the same at pH 5.5 and pH 7. The inhibitory effect of the carbonic acid can also be assigned to the undissociated form.

CONCLUSIONS AND IMPACT OF THE STUDY

The new experimental protocol allowed acquiring data under static conditions in gas phase: the CO_2 dissolution does not modify the partial pressure in head space. Growth rates were acquired in many conditions and finally, a global model was proposed to estimate the growth rate according to the pH, the temperature and the CO_2 concentration in the gas phase.

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Comparative simulation of Escherichia coli O157:H7 behaviour in packaged fresh-cut lettuce distributed in a typical Canadian cold chain in the summer and winter

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OBJECTIVE

Several outbreaks of illness caused by the human pathogen *Escherichia coli* O157:H7 have been associated with the consumption of packaged fresh-cut lettuce (Lynch et al., 2009). This highly perishable commodity should ideally be stored below 4 °C to maintain overall organoleptic quality (Jacxsens et al., 2002). The effect of temperature fluctuations on *E. coli* O157:H7 populations in packaged fresh-cut lettuce and their implication for the risk to public health are not well understood. A recent study performed in Canada showed that conditions conducive to either growth or death were encountered at different stages along a retail supply chain that included storage at the processing facility, distribution centres and retail outlets and transportation between each stage (McKellar et al., 2012). The temperature profiles used in that study were collected during winter months. We have now expanded on the previous work, and report here on differences in temperature profiles measured in winter and summer months in a commercial retail supply chain, and their predicted impact on the fate of *E. coli* O157:H7 in packaged fresh-cut lettuce.

METHODS

Nine cases of ready-to-eat baby leaf lettuce containing six 454 g clam shells were followed from processing to retail in four separate trials carried out during each of two seasons (January/February 2010 and July/August 2011). Lettuce temperatures were recorded in one instrumented clam shell per case with hypodermic thermocouple probes connected to miniature temperature recorders. The thermocouples were imbedded in the stem of a lettuce leaf in the centre of the shells immediately after processing and temperatures were recorded at 5-minute intervals. Distributions were fitted to pooled temperature data for each season using @RISK™ 6.1 in Microsoft® Excel. The total time spent by each case of product at each stage of shipping and storage was also pooled and described by empirical cumulative distributions. @RISK simulations were done using the growth/death model for *E. coli* O157:H7 described by McKellar and Delaquis (2011).

RESULTS

Stochastic simulations were performed to compare the output (log change in cell numbers) from the winter and summer time and temperature distributions. The winter output distribution had an extended left-hand tail, reflecting the greater proportion of low temperatures and longer times in the winter supply chain, resulting in greater die-off. The summer output was more symmetric, and was shifted to the right, relative to the winter distribution, indicating greater potential for growth. Analytical simulations based on individual cases of lettuce under winter and summer conditions using recorded time-temperature profiles were identical to stochastic simulations.

CONCLUSIONS AND IMPACT OF THE STUDY

The output from the model demonstrates a range of possible outcomes, based on experimental times and

temperatures, in a representative fresh-cut lettuce supply chain. The temperature profiles indicated that there was limited temperature abuse during either season, resulting in only slight changes in the predicted pathogen concentration. Nonetheless, temperatures during storage at processor and retail during the summer season enhanced the probability of growth suggesting that attention should be focused on these stages in order to mitigate the risk posed by *E. coli* O157:H7. The distributions present in the current model can be incorporated into QMRA models to improve predictions of levels of the pathogen and probability of illness in fresh-cut lettuce supply chains.

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Comparison of ergosterol content and radial colonies measurements for Byssochlamys fulva and Neosartorya fischeri growth under isothermal conditions

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OBJECTIVE

The most common method used to assess fungal growth in solid substrates is the radial growth of the colonies. However, this method is difficult to be applied for real food substrates. Alternatively, ergosterol content has been used to quantify fungal contamination in food due to it is the dominant sterol in most fungi and it is not found in any significant extent in plants, animals or bacteria. Thus, its quantification in food samples can be taken as an index of the presence of fungi. The aim of this work was modeling the growth of heat resistant fungi *Byssochlamys fulva* and *Neosartorya fischeri* by the methods of measuring the radial growth of the colonies and the ergosterol content using Baranyi and Roberts model [1] to verify the influence of the temperature on the growth kinetics of these fungi on solidified apple juice.

METHODS

The growth medium was prepared from concentrated and clarified apple juice (70 °Brix) added by distilled water until 12 °Brix (soluble solids) and by agar (1.5% in mass). This medium was heated and maintained at 115 °C for 1 min, and then placed in 150 mm Petri dishes. A loop of the analyzed microorganism (10^5 spores/mL) was inoculated into the center of each plate and incubated at 15, 20, 25, and 30 °C. The radial growth of the colonies was measured daily, and the ergosterol content was quantified by HPLC when the colonies had reached 30, 60, 90, 120 and 150 mm [2].

RESULTS

The results showed that *B. fulva* and *N. fischeri* were able to grow very well on solidified apple juice for all temperatures (15, 20, 25, and 30 °C). The Baranyi and Roberts model showed good ability to describe the radial growth of the colonies and the ergosterol content for *B. fulva* and *N. fischeri* on solidified apple juice. A linear trend between the radial growth of the colonies and the ergosterol content for both microorganisms were observed. The ergosterol content showed to be a reliable and useful alternative to assess the fungal growth, especially when the growth in food substrates cannot be assessed by the radial method. Furthermore, the high sensitivity of the ergosterol measurements makes it extremely useful for the quantification of fungi in food, and thus, to ensure the food quality and safety.

CONCLUSIONS AND IMPACT OF THE STUDY

The fungal growth on solidified apple juice was successfully described by Baranyi and Roberts model, which was fitted to measurements of radial growth and ergosterol content. A linear trend between these measurement methods was found, showing that ergosterol content would be a suitable and useful alternative to assess the fungal growth. Therefore, this work can bring a notable contribution for the juice processing industry, since from the data and growth models obtained, it may be established the conditions to avoid the fungal growth in apple juice.

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Investigation of factors affecting oxidoreduction potential of mayonnaise for the setting-up of challenge test procedures

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OBJECTIVE

Predictive microbiology contributes to enhance food quality and safety through a better understanding of microbial behavior in food products. While quantifying and predicting the evolution of a given microflora in a specific food matrix is possible with regard to changes in pH, temperature, water activity and organic acid concentration, there is very little information on the bacterial response to modifications of oxidoreduction potential (E_h). However, previous studies showed that this parameter influences many cellular functions[1] and impacts the overall quality of food products[2].

The French national research program Food-Redox (2012-2014)* intends to use E_h as a new hurdle to better control microbial contaminations in the products and processes of food industries. Notably, it aims at integrating this parameter in food models for their fine-tuning and improvement. For that purpose, a prerequisite is to determine the means and conditions for E_h control in food matrices before implementation in challenge-tests for data collection. The present work investigates the levers enabling to control E_h in mayonnaise for subsequent evaluation of E_h levels on growth parameters of typical pathogen and spoilage bacteria encountered in such acidified sauce.

METHODS

Formulation and process parameters likely to have an impact on E_h levels in mayonnaise were screened and selected based on in-depth literature review. The effective influence of these parameters on E_h was assessed using design of experiments (DoE) methodology. Pilot scale preparations of DoE formulations were tested in different atmosphere conditions and their E_h and pH were simultaneously measured using combined electrodes. The pH dependency of E_h in mayonnaise was evaluated experimentally in each studied atmosphere condition using the average formulation of the DoE. This enabled to calculate the E_h-pH correlation factor of the Leistner and Mirna equation[3] to overcome pH dependency (when appropriate).

RESULTS

The selected factors could be discriminated according to their relative influence on E_h, whether alone or in interaction. While, as expected, the atmosphere showed a significant ($p<0.05$) impact on E_h, some ingredients like potassium sorbate, vinegar, sugar and salt also displayed significant ($p<0.05$) effects on this parameter. Further statistical analysis of DoE data enabled to ascertain a preliminary model of E_h as a function of the concentration of these ingredients and the type of atmosphere. This model can serve to control E_h levels in mayonnaise.

CONCLUSIONS AND IMPACT OF THE STUDY

By applying a specific approach based on DoE, this work provides proof of concept that it is possible to identify the factors affecting E_h in food matrices and to evaluate their respective contribution as well as their interaction. This will help setting-up challenge test procedures to study the impact of E_h levels on the

behavior of pathogenic and spoilage bacteria in food products.

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Development and validation of an extensive growth model for Lactobacillus spp. in seafood and meat products

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OBJECTIVE

The objective of the present study was to model and predict growth of *Lactobacillus* spp. in seafood and meat products.

METHODS

An existing cardinal parameter model for growth of lactic acid bacteria (LAB) in seafood products (Mejlholm and Dalgaard, 2007) was expanded with the effect of nitrite, and acetic, benzoic, citric and sorbic acids to include a total of 12 environmental parameters as well as their interactive effects (Le Marc et al., 2002). Firstly, to estimate values for μ_{ref} and T_{min}, the existing LAB model was refitted to data from 96 experiments with seafood and meat products not including nitrite or any of the four evaluated organic acids. Secondly, dimensionless terms modelling the antimicrobial effect of nitrite, and acetic, benzoic, citric and sorbic acids on growth of *Lactobacillus sakei* were added to the refitted model, together with minimum inhibitory concentrations determined for the five environmental parameters. For model validation, 58 experiments with seafood products were carried out. In addition, data from 262 experiments studying the growth of *Lactobacillus* spp., *Carnobacterium* spp., *Leuconostoc* spp. and *Weissella* spp. in seafood and meat products were collected from the literature.

RESULTS

The new model was successfully validated for 229 growth kinetics of primarily *L. sakei* and *L. curvatus* in seafood and meat products. Average bias and accuracy factor values of 1.08 and 1.27 were obtained when observed and predicted growth rates (μ_{max} values) of *Lactobacillus* spp. were compared. Thus, on average μ_{max} values were only overestimated by 8%. The performance of the new model was equally good for seafood and meat products, and the importance of including the effect of acetic, benzoic, citric and sorbic acids and to a lesser extent nitrite in order to accurately predict growth of *Lactobacillus* spp. was clearly demonstrated. For the most preserving combinations of product characteristics and storage conditions the performance of the new model was clearly improved by including the effect of interactions between the environmental parameters. On average, μ_{max} values of *Carnobacterium* spp., *Leuconostoc* spp. and *Weissella* spp. were overestimated by 35 to 38% by the new model.

CONCLUSIONS AND IMPACT OF THE STUDY

In the present study, an extensive growth model for *Lactobacillus* spp. was successfully developed and validated. It was concluded that a single predictive model is sufficient to accurately predict growth of *Lactobacillus* spp. as long as its complexity match the complexity of the products (i.e. all the important environmental parameters should be included). The new model can be used to predict growth of *Lactobacillus* spp. in seafood and meat products e.g. the time to a critical cell concentration of bacteria being useful for establishing the shelf life. In addition, the high number of environmental parameters included in the new model makes it flexible and suitable for product development as the effect of substituting one combination of preservatives with another can be predicted. Furthermore the new model can be used in combination with models for e.g. *Listeria monocytogenes* in order to predict the importance of microbial

interactions on the maximum population density of pathogens e.g. in connection with risk assessment studies. Although not directly applicable for *Carnobacterium* spp., *Leuconostoc* spp. and *Weissella* spp., the new model provided interesting information on their growth characteristics in comparison to *Lactobacillus* spp. In the future it seems interesting to include the use of accurate predictive models in studies on the microbial diversity of foods as differences in product characteristics and storage conditions are likely to have a major effect on the observed biodiversity.

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Modelling the thermal resistance of starvation-stressed Salmonella spp. in ground chicken as affected by cinnamaldehyde and carvacrol

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OBJECTIVE(S)

It has been previously observed that supplementing chicken meat with 0.1 to 1.0% trans-cinnamaldehyde (the main ingredient in cinnamon oil widely used as a spice in many foods) or carvacrol (the main ingredient in oregano essential oil used as a salad dressing) rendered *Salmonella* more sensitive to the lethal effect of heat (Juneja et al, 2012). On the other hand, starvation stress of *Salmonella* is likely to occur in the poultry processing plant as the cleansing water provides a nutrient poor environment for the cells. Thus, the objectives of this study were to model (i) the combined effects of cinnamaldehyde, carvacrol and temperature on the thermal inactivation of starved *Salmonella* cells in ground chicken; and (ii) the time needed to obtain a 7.0 log relative reduction (t_{70}) as required by current performance standards for the commercial processing of partially cooked poultry.

METHODS

A complete factorial design (3 x 4 x 4), consisting of three internal temperatures (60, 65 and 71.1°C) and four concentrations (0.0, 0.1, 0.5 and 1% vol/wt) of the two natural antimicrobials, was used to assess the heat resistance of an eight-strain cocktail of starvation-stressed *Salmonella* serovars inoculated in chicken meat. The 48 variable combinations were replicated to provide a total of 96 survivor curves from the experimental data. To mathematically characterise the combined effects of these parameters on the heat resistance of starved *Salmonella* cells, an omnibus log-linear model with tail and an omnibus empirical sigmoid model were fitted to the entire data set integrating primary and secondary models. Other secondary models to predict t_{70} and the D-value as functions of the environmental conditions in the ground chicken were also assessed.

RESULTS

Overall, the omnibus sigmoid model provided a better description of the observed data than the omnibus log-linear model with tail. However, both models showed that the addition of the plant-derived antimicrobials in ground chicken decreases considerably the heat resistance of starvation-stressed *Salmonella* cells; and that the higher the temperature, the greater the effects of the cinnamaldehyde and carvacrol concentrations on the inactivation rate. Predictions of the t_{70} based on the log-linear model were in most cases smaller than those based on the sigmoid model because of the upward concavity of the inactivation curves; and the greater differences between the two predictions occurred for the milder conditions of lower heating temperatures and lower concentrations of antimicrobials. The expression for the D-value, extracted from the omnibus log-linear model, predicted within the 95% confidence interval other *Salmonella* D-values reported in the literature for chicken meat; despite differences in *Salmonella* serovars and recovery media. In some cases, there was an overestimation of D-values due to the use of (more

resistant) starvation-stressed *Salmonella* cells.

CONCLUSIONS AND IMPACT OF THE STUDY

The addition of plant-derived antimicrobials such as carvacrol and cinnamaldehyde reduces the heat resistance of starvation-stressed *Salmonella* in ground chicken. This beneficial effect of the antimicrobials enables heat treatments to be reduced to allow achieving the same safety objectives and to retain nutritional quality of ground-chicken products. The discrepancy among the two models only became apparent when lethality times from each of the models were calculated; and hence the omnibus sigmoid model was preferred. Because published studies show that carvacrol and cinnamaldehyde act as broad-spectrum antibiotics against *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* in laboratory media, it would be of interest to develop other survival models to predict the antimicrobial effects against multiple food-borne pathogens in different meat and poultry products.

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Modeling non-isothermal heat inactivation of Bacillus coagulans spores in tomato pulp

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OBJECTIVE

Canned fruit and vegetables have been widely consumed for decades during seasons of the year when fresh foods are not available. These products are acidified and/or given thermal processes to render them shelf stable. However, spore-forming microorganisms such as *Bacillus coagulans* can tolerate such treatments and proliferate in the products causing spoilage and potentially great economic losses to the industry. Thermal processes are efficient at reducing and/or eliminating microorganisms and the efficacy of this process is generally assumed when the temperature profile is static. However, processing temperature fluctuation can occur during daily operations in the food industry and this fluctuation may be extensive. The heat applied in the "come-up time" during thermal processes is usually not considered in microorganism heat resistance calculations. Therefore, the knowledge and understanding of the microorganisms inactivation during a thermal treatment, as influenced by temperature variation during the thermal processes, are essential for design, calculation and optimization of the process.The objectives of this study were: (i) to predict inactivation kinetics of *B. coagulans* as influenced by different dynamic temperature profiles using dynamic model proposed by Peleg and Penchina (2000), and (ii) to validate the model comparing the predict values with experimental data obtained through *B. coagulans* inactivation in tomato pulp under identical realistic temperature profiles.

METHODS

Assuming that the momentary inactivation rate is the isothermal rate at the momentary temperature at a time that corresponds to the momentary survival ratio, the survival curves under non-isothermal conditions could be constructed by solving a differential equation, as proposed by Peleg and Penchina (2000). Experimental inactivation data of *B. coagulans* spores in tomato pulp using capillary tube were obtained at seven different dynamic temperature profiles, in the range from 95 to 105 °C, to validate the model prediction.

RESULTS

Predicted inactivation kinetics by the model showed similar behavior to experimentally observed inactivation kinetics for a temperature range from 99 to 105 °C. Profiles that resulted in less accurate predictions are those where the range of temperatures analyzed were lower (inactivation profiles starting at 95 °C), which represent the lower level in the studied range. Overall model predictions were acceptable, with bias factors from 0.781 to 1.012, and accuracy factors from 1.049 to 1.351. The non-isothermal temperature profiles in this study were similar to those observed under actual processing conditions, and confirms that the models used were adequate to predict *B. coagulans* spores inactivation under fluctuating temperature conditions in the range of 95 to 105 °C.

CONCLUSIONS AND IMPACT OF THE STUDY

The non-isothermal model predictions were similar to those observed under actual processing conditions, validating and confirming that the mathematical procedure proposed by Peleg and Penchina (2000) is adequate to estimate *B. coagulans* spores inactivation. Non-isothermal simulations can help to evaluate the

efficacy of remedial measures in industry processes.

Keywords: *Bacillus coagulans*, predictive microbiology, non-isothermal inactivation, dynamic model.

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Modelling complex microbial interactions: Listeria monocytogenes in soft ripened cheese

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OBJECTIVE

The subject of this modelling study is the manufacturing of soft ripened cheese considering the most relevant biochemical reactions in the cheese ecosystem. The model must be dynamic (technically: described by differential equations) since a series of interactions between bacterial and chemical elements change this ecosystem considerably during maturation. A rich literature is available for the participating biochemical reactions but here it is considered a largely reduced version only, for a quick and straightforward application to predictive modelling in food safety.

In this simplified model, the system has three microbial variables: log concentrations of *L.monocytogenes*, lactic acid bacteria and mould, and environmental/chemical variables: temperature, pH and protease concentration.

As a first step to mechanistic modelling, the initial time of observation and the values of the considered variables at this initial time (initial values) need to be defined. The next step is to identify (i) intrinsic and (ii) extrinsic parameters and (possibly time-dependent) variables. The latter ones are set up by the user, and the dynamics of the studied system does not affect them. However, the intrinsic variables do affect each other, making the system "live".

Therefore the objective is to develop a relatively simple dynamic model that is still sophisticated enough to capture the main features of the temporal variation of the cheese ecosystem and predict the behavior of *Listeria monocytogenes* inoculated in it.

METHODS

Different challenge test studies were performed to monitor *L.monocytogenes* concentration in soft ripened cheeses, in particular blue veined and washed rind cheeses. *L.monocytogenes* strains were inoculated in different steps of cheese process. The indigenous (lactic acid bacteria and mould) and inoculated (*L.monocytogenes*) log cell concentrations as well as intrinsic parameters, pH and a_w, were measured during the process, at various time points.

The used kinetic equations and the needed biochemical rates were partly from the literature, partly derived from own measurements. The data were recorded in Microsoft Excel spreadsheets and, for the sake of simplicity, a Runge-Kutta 2nd order solver was programmed on the spot (*i.e.* in the spreadsheet), to simulate the developed model.

RESULTS

The initial values were obviously extrinsic parameters. The temperature is not affected by the dynamics of the system, therefore it (and its temporal variation) was also considered extrinsic. It has a control role, to determine the rates of the intrinsic biochemical reactions. The variables in the differential equations were the

three microbial log concentrations and the two chemical variables.

We assumed that the rate coefficients depended on the temperature only and they instantaneously take up the value determined by the actual temperature. This is a simplifying assumption, just as the "short cut" by which a reaction equation was considered in one step even if known that it is the result of several intermediate interactions.

The resultant model was validated by observations at a dynamic temperature profile. The obtained simulation captured the expected features: the lactic acid produced by lactic acid bacteria was metabolized by mould, which produced protease, with ammonia release, which increased the pH. This, in return, allowed *Listeria* to grow. A quantified prediction of its growth was the final objective of the study. The validation experiment showed a reasonable agreement with observed data.

CONCLUSIONS AND IMPACT OF THE STUDY

Predictive modelling is still and will remain a primarily empirical discipline. However, whenever possible, mechanistic elements should be included in the modelling method, linking it to fundamental sciences. Modelling complex microbial interactions will be a prime area requiring such approach.

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Evaluation of the inactivation of Salmonella enteritidis in mayonnaise supplemented with oregano essential oil

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OBJECTIVE(S)

The aims of this study were:
a) to investigate the effect of oregano essential oil on the survival of *Salmonella enteritidis* in home-made mayonnaise;
b) to evaluate the Baranyi model for modeling survival curves;
c) to obtain a secondary model that expresses the specific growth rate as a function of the concentration of oregano essential oil.

METHODS(S)

Preparation of inoculum
A strain of *Salmonella enteritidis* ATCC 13076 was provided by Professor W. A. Padilha (Federal University of Pelotas, Brazil), and was maintained on slopes of nutrient agar at 7ºC. An overnight culture was grown in Brain Heart Infusion as culture medium at 36ºC. It was used commercial oregano essential oil (Lazslo, Brazil). For the inoculation of the samples, 1 ml of the suspension was used.

Enumeration of microorganisms
For the enumeration of *Salmonella enteritidis*, a 10g sample of the mayonnaise was suspended in 90ml of peptonated water. A 9 ml aliquot of the appropriate dilution was spread on duplicate plates of Brilliant Green Agar *Salmonella sp.* and incubated at 36ºC for 24h.

Mayonnaise preparation
Mayonnaise was prepared according to the following recipe: 175 ml of soybean oil, 17.5 g of yolk, 9 ml of sodium chloride, 1.25 ml of EDTA, and 25 ml of vinegar. The ingredients were mixed using a commercial blender.

Mathematical modeling
At each concentration of oregano essential oil (0.0%, 0.7%, 1.4%), 6 bacterial points were modeled as a function of time. Initial inoculum was fixed at 6 log counts and pH was fixed at 4.0. For fitting purposes, the model of Baranyi and Roberts (1994) was fitted to the logarithm of bacterial concentration. For curve fitting, the program DMFit 2.1 (Institute of Food Research, Reading, UK) was used.

RESULTS

In the present study, as expected, the addition of oregano essential oil to mayonnaise resulted in a decrease in the number of *Salmonella enteritidis* after inoculation (Baj Pai et al., 2012). However, as can be seen in Figure 1, this decrease was also observed for the control sample (0.0 %) which can be attributed to the high acidity of the mayonnaise. Thus, it was observed an additive effect between pH and oregano, which was also observed in other study (Koutsoumanis et al., 1999). The results obtained for the death rate were, respectively, 0.2397 (0.0% of oregano essential oil), 0.2661 (0.7%), and 0.4153 (1.4%). The following secondary model was derived for the relation between the death rate and the concentration of oregano

essential oil [*OEO*]: $m_m = 0.2192 + 0.1254[OEO]$ ($R^2 = 0.86$). The results obtained indicated that for a 0.1% increase of oregano essential oil, it corresponds a 0.0125 increase of the death rate.

CONCLUSIONS AND IMPACT OF THE STUDY

This study was directly conducted in a food system and consequently, is able to produce results specific to the design of mayonnaise. In addition, it allows the quantitative evaluation of oregano essential oil as a natural antimicrobial.

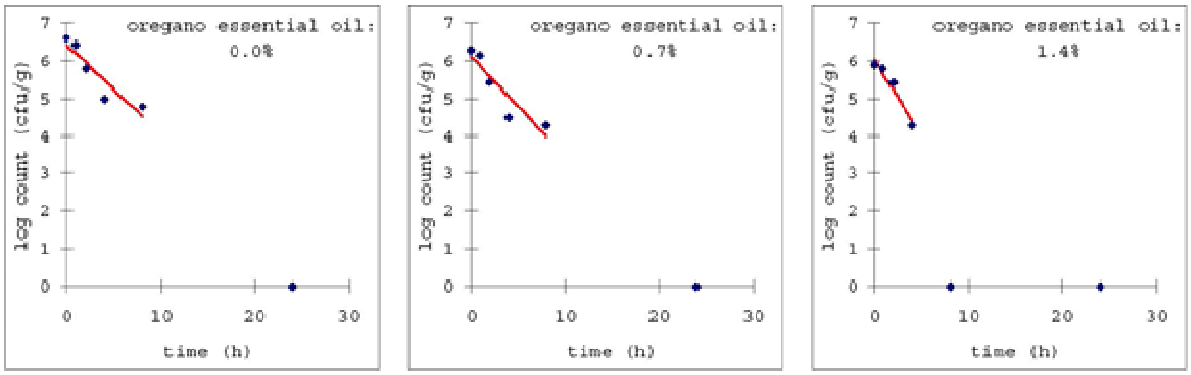

Figure 1: Inactivation curves for *Salmonella enteritidis*, fitted with the Baranyi model, in mayonnaise with pH 4.0 supplemented with different concentrations of oregano essential oil. Determination Coefficient: $R^2 = 0.795$ (0.0% of oil), $R^2 = 0.775$ (0.7% of oil), $R^2 = 0.921$ (1.4% of oil).

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Physiological traits of fungal and yeast species: a predictive mycology approach

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OBJECTIVES

Moulds and yeasts are micro-eukaryotes of food interest insofar they are used as processing aids but they are also responsible for alterations of food and initiate important economic losses. Despite their large implication in food industry, very few studies have been conducted to quantify the growth of the main fungal dairy spoilage or fungal or yeast processing aid species. In the present study, predictive modelling was used to reflect the effects of temperature, pH, and water activity (a_w) on the growth of mould and yeast species.

METHODS

One strain of seven fungal species commonly considered as contaminants of dairy products, nine fungal processing aid species and five yeast processing aid species were studied. Effects of three abiotic factors on fungal and yeast growth were individually investigated: temperature (9 levels from 3 to 45°C), pH (buffered media, 8 levels from 2 to 12) and a_w (10 levels from 0.82 to 0.99). Fungal culture was carried out up to 35 mm radius for 10 weeks maximum on Potato Dextrose Agar medium. Diameters were measured in two orthogonal positions and the mean radius was plotted against time. Yeast growth was carried out in Sabouraud dextrose broth and followed by UFC count on Sabouraud dextrose agar medium. The growth kinetics were fitted by four primary models. Based on quality of fit, meaning of the estimated parameters, the most convenient model was chosen. Then, the evolution of the primary parameters was fitted by models with physiological meaning parameters.

RESULTS

The effects of temperature and water activity on the growth rate were fitted by cardinal values models for the sixteen fungal strains and five yeast strains.

The study of the temperature indicates that some species (*Aspergillus flavus*, *Byssochlamys nivea* and *Mucor circinelloïdes*) tolerate high temperature (from 37°C to 43°C) and all others species (including *M. circinelloïdes*) withstand low temperatures (above 5°C). According to the species, the optimum temperature is between 20 and 35°C with an optimal growth rate ranged from 0.5 to 12 mm/day. For yeasts (*Debaryomyces hansenii*, *Kluyveromyces lactis*, *K. marxianus*, *Saccharomyces cerevesiae* and *Yarrowia lipolytica*), the minimum temperature is between 0 and 3°C, the optimal temperature between 31 and 37°C and the maximum temperature drops very rapidly between 37 and 40°C.

Concerning a_w, *A. flavus*, *Penicillium brevicompactum*, *P. camemberti*, *P. fuscoglaucum*, *P. nalgiovense*, *P. roqueforti* and *P. solitum* were able to grow at 0.82. The growth of the other species started to be observed at 0.88. According to the species, the optimum a_w is between 0.96 and 0.99. Yeasts seem to be more sensitive to the effect of low a_w. Indeed, yeasts grow at a_w between 0.90 and 0.93.

The response of fungi to pH changes is highly variable. Some species show a clear optimum (*A. flavus*, *Cladosporium herbarum*, *M. circinelloïdes*, and *P. expansum*). Others species show a linear effect of the pH on the growth rate (*B. nivea*, *Fusarium domesticum* and *Sporendonema casei*). And four *Penicillium* species are insensitive to pH changes. All fungal species can grow at pH 4.0. pH_{max} differed from species and was between 8.0 and 9.0 for most of the *Penicillium* species. The yeast growth trends are more homogenous with a pH_{min} between 1 and 2.5 and maximum pH_{max} between 9 and 12.

CONCLUSIONS AND IMPACT OF THE STUDY

The results define the eco-physiological requirements of these fungal spoilers and fungal and yeasts processing aids have an evident interest to understand, quantify and control their behaviour in dairy products. A software tool was proposed in order to rank these fungi and yeasts as a function of their growth potential for a given time and given conditions of storage.

Shelf life modelling of osmotically dehydrated chicken breast fillets during refrigerated storage

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OBJECTIVE

Osmotic dehydration of food products has received increased attention as a pretreatment to further processing. Most studies have been conducted on plant tissue and the potential for shelf-life extension has been demonstrated. For meat products, fewer references are available. Over the past decade, there has been a steady increase in the demand for convenient cuts of poultry meat. Chicken breast fillets are of high interest but suffer from shorter shelf life. Minimal processing by osmotic dehydration could significantly extend shelf life. The objective of the study was to evaluate the effect of osmotic dehydration on the shelf life of chicken breast fillets during refrigerated storage.

METHODS

Sliced samples (cubes, 10±1g) were treated with 50% maltodextrin (DE47) plus 5%NaCl for 30 min at 15°C. HDM/NaCl solution with 0.5% glucono-δ-lactone was also used. Untreated and pretreated slices were aerobically packed and stored isothermally (0, 5, 10 and 15°C). Quality assessment was based on microbial growth (TVC, *Pseudomonas* spp., lactobacilli), pH, colour, texture and sensory scoring. The microbial growth was modelled using the Baranyi Growth Model (Baranyi & Roberts, 1995). For curve fitting the in-house program DMfit of IFR (Institute of Food Research, Reading, UK) was used. Kinetic parameters such as the rate (k) of the microbial growth were estimated. Quality indices were kinetically modelled and temperature dependence of quality loss rates was modelled by Arrhenius equation. Analysis of variance (ANOVA) at a significance level of 95% was used for the analysis of quality degradation rates of untreated and osmotically treated chicken breast fillets (STATISTICA® 7.0, StatSoft Inc., Tulsa, USA). Significant differences were calculated according to Duncan's multiple range test ($a = 0.05$).

RESULTS

Osmotic pretreatment resulted in a water activity decrease to 0,96. Microbial growth and sensory changes were modelled at isothermal conditions and the temperature dependence of each index was expressed by the E_a value of the Arrhenius equation (ranging from 55 to 87 kJ/mol). End of shelf life determined by sensorial unacceptability was correlated to a *Pseudomonas* level of 8 logCFU/g. Osmotic pretreatment led to significant shelf life extension of chicken, in terms of microbial growth and organoleptic deterioration. The use of antimicrobial agents gave additional shelf life increase of pretreated chicken. Based on sensory acceptability, the shelf life was 5 d for raw samples and 8 d for osmotically pretreated chicken at 5°C. The additions of glucono-δ-lactone increased the shelf life to 11 d at 5°C.

CONCLUSIONS AND IMPACT OF THE STUDY

The objective of the study was to evaluate the effect of osmotic pretreatment on the quality characteristics of chicken breast fillets and to investigate the potential of using osmosis as a minimal treatment to extend the shelf life of poultry products. Pretreated samples were found to have improved quality stability during subsequent refrigerated storage, in terms of microbial growth and sensory changes, resulting in a significant shelf life extension at all storage temperatures. The results of the study show the potential of adding glucono-

δ-lactone in the osmotic solution to further extend the shelf life and improve the commercial value of chilled osmotically pretreated chicken breast fillets. The developed models can be a reliable tool for predicting the shelf life of fresh or minimally processed chicken breast fillets fillets in the real chill chain.

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Modeling of the inhibition of Listeria monocytogenes by Enterococcus faecium

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OBJECTIVE(S)

The aims of this study were:
a) to investigate the inhibition of *Listeria monocytogenes* by *Enterococcus faecium*;
b) to evaluate the Baranyi model for modeling the resulting growth curves.

METHODS(S)

Preparation of inoculum
A strain of *Listeria monocytogenes* Scott A was provided by Professor W. A. Padilha (Federal University of Pelotas, Brazil) and *Enterococcus faecium* was isolated from raw milk in Pinhalzinho, SC, Brazil. Both strains were maintained on Trypticase soy agar at 7ºC.

Enumeration of microorganisms
Pure cell suspensions of *Listeria monocytogenes* and *Enterococcus faecium* were used as controls at 36ºC. Cocultivation experiments were carried out by mixing adequate portions of cultures of *L. monocytogenes* and *E. faecium* in Brain Heart Infusion (BHI) broth at 36ºC for 18h. Dilutions were plated on BHI agar supplemented with lithium chloride at 1.5% for viable counts of *Listeria monocytogenes* and on Rothe agar for viable counts of *Enterococcus faecium* (Mendoza et al., 1999). All measurements were duplicated.

Mathematical modeling
At each of the three growth curves (pure *Listeria monocytogenes*, pure *Enterococcus faecium*, and coculture), 13 bacterial points were modeled as a function of time. Initial log counts used in this study were of 5.7 for pure *L. monocytogenes*, 6.0 for pure *E. faecium*, and 4.6 for both strains at the coculture. For fitting purposes, the model of Baranyi and Roberts (1994) was fitted to the logarithm of bacterial concentration. For curve fitting, the program DMFit 2.1 (Institute of Food Research, Reading, UK) was used.

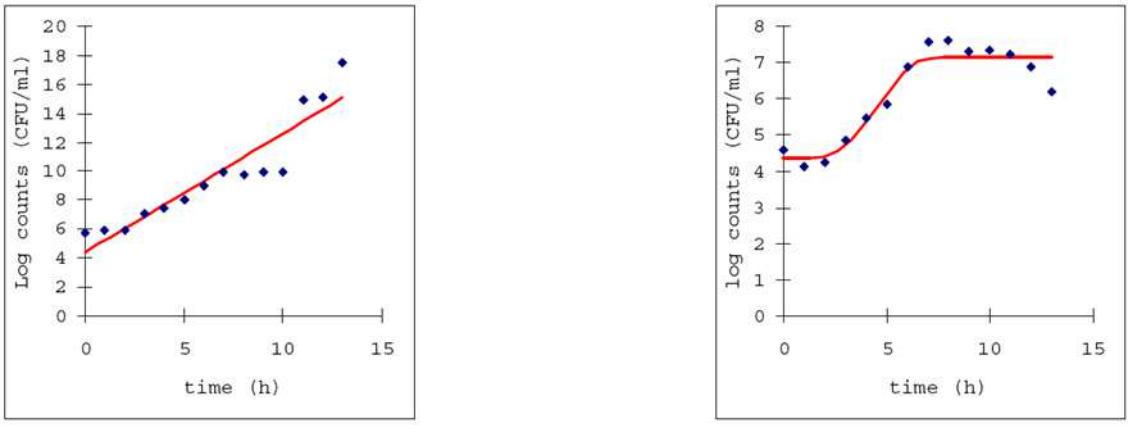
RESULTS

Figure 1 presents the growth curves obtained for *L. monocytogenes* without (curve a) and with (curve b) addition of *E. faecium*, respectively. The growth rate obtained for the pure sample of *Listeria monocytogenes* was 0.8220 and for the coculture, the estimated growth rate was 0.8968. It was observed that immediately after inoculation, the presence of *Enterococcus faecium* did not affect the growth of *L. monocytogenes* and, as can be seen from Figure 1, after approximately 9 hours of inoculation, it was observed a decrease of the number of *Listeria monocytogenes*. Another study revealed that no inhibition of listeria growth was observed before 6 hours of inoculation because of the small production of bacteriocin in the early stages of *E. faecium* growth (García et al., 2004). The numbers of enterococcus did not decrease during the experiment (not shown).

CONCLUSIONS AND IMPACT OF THE STUDY

The use of biopreservation methods in the control of listeria and other pathogens is one topic of great concern in the food industry. In this context, bacteriocinogenic strains of enterococci can be used as a good alternative to bacteriocin addition in food systems. Thus, this study aims to contribute to the study of the

inhibition of *L. monocytogenes* by *E. faecium* in deeper details



(a) (b)

Figure 1: a) Growth curve for pure *Listeria monocytogenes*, fitted with the Baranyi model. Determination Coefficient: $R^2 = 0.859$. b) Growth curve for *Listeria monocytogenes* added with *Enterococcus faecium*, fitted with the Baranyi model. Determination Coefficient: $R^2 = 0.897$.

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Predictive modeling to determine the time for deterioration of single strength orange juice, pH 4

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OBJECTIVE(S)

This research aimed to model the time for 1% deterioration of refrigerated orange juice as a function of temperature and inoculum level, intentionally inoculated with *Mrakia frigida*, psychrophilic yeast isolated from product.

METHODS(S)

Microorganisms and spore suspension production

The spore suspension of *Mrakia frigida* was produced from previous inoculum in PDA (15°C/7days), following which it was suspended in sterile water and stored at(2º). A 0.2mL aliquote of suspension was transferred to Roux bottles containing Yeast Malt Extract Agar (YMA, formulated, pH 5.5) added of manganese sulfate solution 5ppm; incubated at 15°C/30days. Sporulation was monitored until 90% of spore production was observed, spore collection was performed and centrifugations were conducted at 4°C/10min /3000rpm. Suspension was transferred to a sterile bottle and stored at 4°C for future use.

Inoculation, Incubation and data collection

Spore suspension was adjusted for a final concentration of 10^1 and 10^2spores/mL and inoculated in 30 bottles/assay PET bottles, containing 350mL of sterile orange juice incubation temperatures were 2, 4 and 10°C. Bottles were daily monitored for the presence of gas in inverted TDT sterile tubes allocated inside the juice bottles and bottle distension. Data collection of time for gas bobble observation or gas distention of the bottle were used and the logistic model adjusted to determine the time for 1% of orange juice samples to show spoilage and for estimation of **K**, **T** and Pmax, Whiting & Call (1993):

$$P = \frac{P\max}{1+\exp^{\kappa(\tau-t)}} \qquad \text{Eq.1}$$

$$t = \tau - (\ln(P\max/P) - 1)/\kappa \qquad \text{Eq.2}$$

Where: P = probability of deterioration as a function of time (%); t = time (days); Pmax = maximum probability of spoiled bottles (%); **K** = slope parameter (day^1); **x** = time, days, for P=Pmax/2.

RESULTS

Regardless of tested temperature, for 10^2 inoculated spores/mL the number of spoilage bottles and the % of spoilage were higher than for 101spores/mL. At 4°C, 10^2spores inoculum/mL was observed the minimum deterioration time (1 bottle spoiled/30 bottle inoculated), in 3 days. Maximum deterioration period, 68 days, was observed for 2°C for both inoculums and 4°C for 101spores/mL. Figure 1 shows the accumulated percent of spoiled bottles x time for 10^1(Fig.1a) and 10^2spores/mL(Fig.1b).

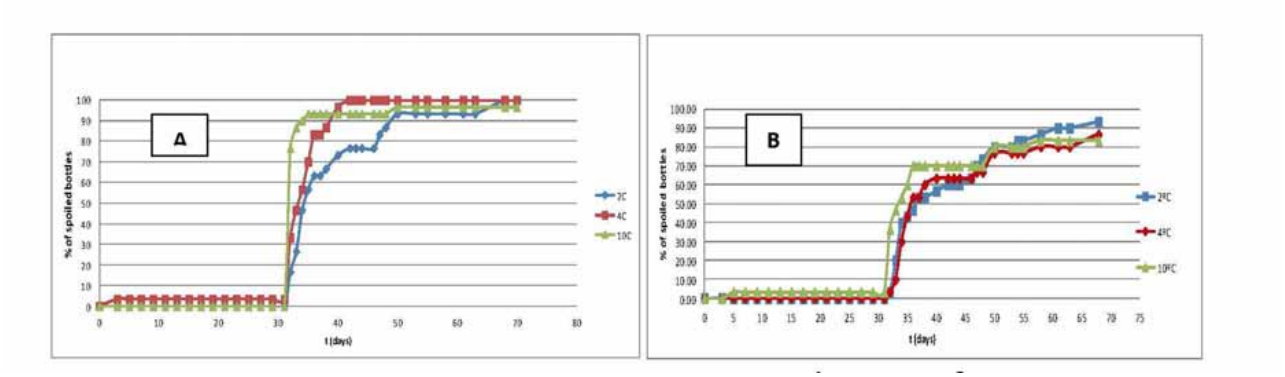

Figure 1. Accumulated percent of spoiled bottles x time for 10^1(A) and 10^2spores/mL(B).

The maximum probability of spoilage, obtained from logistic model, was 99.43% at 4°C/10^2spores/mL (K=0.61 and x=33.57) and the minimum was 72.25% at 4°C/10^1spores/mL (K=0.68 and T=34.88).

Using estimated parameters and Eq.2, it was calculated the time for 1% of bottles showing deterioration (Table 1).

Table 1. Time (days) for 1% deterioration for all tested conditions, according to Whiting & Call (1993).

Inoculum level	*Temperature (°C)*	*Time for 1% of deterioration (days)*
10^1	2	18.26
	4	28.63
	10	26.94
10^2	2	24.77
	4	26.06
	10	31.14

At 2°C the time for initial deterioration decreases probably because this is the best temperature for this organism to develop. The predicted curve does not describe well the adaptation phase but it adjusts better to the exponential phase data. Experimentally, at 2°C deterioration occurred at 32days for 10^1 or 10^2spores/mL and by model, it occurred at 24days, 10^2spores/mL, showing that the model is conservative Fig. (2)

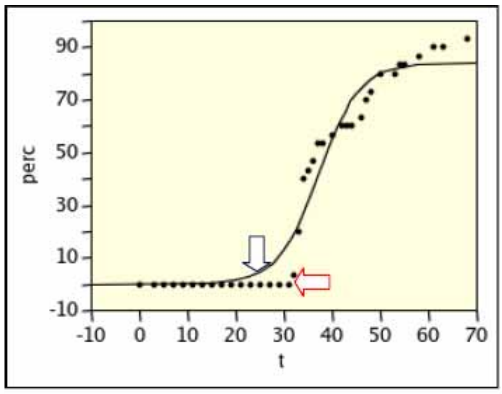

Figure 2. **Whitting & Call (1993) model adjusted to experimental data: T=2°C and 10^2spores/mL.**

CONCLUSIONS AND IMPACT OF THE STUDY

During long distance transportation of refrigerated orange juice predictive models can be use to estimate the time for deterioration of a small portion of the juice, even when a low contamination of a psychrophilic yeast occurs, providing valuable information for the orange juice processors to take corrective action on the destination of such product. Refrigerated storage at 2ºC does not guarantee microbial stability if the juice is contaminated. This is the first time this information is reported.

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Microbiological challenge study: factors affecting target pathogen recovery from food

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OBJECTIVE

Microbiological challenge tests have been useful sources of data in predictive microbiology. According to technical documents on shelf life studies products can be inoculated through the package wall by the use of syringe and rubber septum. Even though this method has many benefits compared to inoculation before packaging or to re-pack after inoculation directly in the food, it may represent a risk of uneven distribution of the target bacteria especially in complex food matrices. The objective of the present study is to evaluate inoculation procedures using *Listeria monocytogenes* as a model organism.

METHODS

The following convenience full meals packed in modified-atmosphere packaging (MAP) were investigated (i) meatball dinner, (ii) fish stew, (iii) chicken stew, and (iv) paella. Inoculations of the products into the MAP were essentially performed following the EU technical guidance document on shelf life studies for *L. monocytogenes*. In brief, cocktails of *L. monocytogenes* strains were aseptically injected using a sterile syringe through the package wall containing a rubber septum mimicking realistic contaminations (on the surface and/or inside the food). A food dye was used to visualize the distribution of inoculum in the MAP packed products. In parallel representative samples were aliquoted into plastic bags from opened packs and artificially contaminated with *L. monocytogenes*. Three levels of *L. monocytogenes* inoculation (low, medium and high) were added. Analyses were performed just after inoculation (day 0). Amount of *L. monocytogenes* added and amount recovered from foods were compared using statistical t-test.

RESULTS

Generally the amount of *L. monocytogenes* recovered from the food matrices was lower than the amount added. *L. monocytogenes* counts from the MAP samples were slightly lower than recovered from the bags, however, the difference was statistically insignificant ($p>0.05$). The dye experiment indicated that the inoculum can be dispersed beyond the inoculated target area. Increasing the sampling surface area and the amount of sample improved the recovery rate of *L. monocytogenes* from the food. Recovery rate of the target bacteria was dependent on amount of inoculation levels and types of food matrices. The EU technical guidance document stated that the variation among the three parallels for the “day 0” samples shall be less than 0.3 log cfu/gm standard deviation. Of the 15 trails using medium level of inoculation, 87% of the variations were below the threshold.

CONCLUSIONS AND IMPACT OF THE STUDY

Septum inoculation technique is logistically less demanding and satisfactory method to study the shelf life of products in special packaging system, provided that spread of inoculum beyond the sampling area is avoided/limited. The data will be used for the development of decision support tools in the EU project (STARTEC).

Determination of Alternaria growth and mycotoxin boundaries in tomato purée model

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OBJECTIVES

Alternaria species were reported to be the most commonly fungi affecting either tomato fruit and plant causing the so called black mold of tomato. Rapid infection of *Alternaria* in tomato may occur on the crop or post harvest yielding high economical losses due to spoilage of industrialized products such as tomato purée. Under optimal growth conditions, *Alternaria spp.* may also produce various mycotoxins. Alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) are mycotoxins commonly found in tomatoes and tomato products, representing a serious risk for human health related to the consumption of these products. This study aims at definying boundaries for growth and mycotoxin production in order to optimize product formulation and shelf-life.

METHODS

A toxigenic isolate of *Alternaria alternata* (ITEM8176) isolated from tomato fruit affected by black mold and deposited at the ISPA collectin, Italy (ITEM accession: http://www.ispa.cnr.it/Collection/) was used for growth and mycotoxin production assessment. Growth ability of the strain was determined after inoculating fungal ascospores (7day-old culture) on tomato purée supplemented with agar and followed by regular fungal development observations. A total of 6 levels of pH and 10 levels of temperature were tested, for 3 replicates, to define pH and temperature boundaries where fungal development and mycotoxin production occurred. The pH of tomato purée based medium was fixed at 2, 3, 4, 5, 6 and 7 while plates incubation was performed at 6.5, 10, 15, 20, 25, 30, 35 and 37°C. Analysis of mycotoxins (AOH, AME and TeA) was performed by HPLC with UV/DAD detection according to an adapted protocol from Solfrizzo *et al.* 2004.

RESULTS

Stability of pH and water activity of tomato purée based media were checked throughout the experiments. Growth was observed above a pH of 3 whatever the incubation temperature. Growth optimum was determined at pH 5.5 and 24.5°C. Conditions where growth was not observed after 1 month incubation were considered as not allowing fungal development as observed for pH lower than Growth/no growth boundaries were compared with mycotoxin production/ no mycotoxin production boundaries for similar conditions on tomato purée based medium.

CONCLUSIONS AND IMPACT OF THE STUDY

These results indicate the combination of pH and temperature where *Alternaria* molds development and mycotoxin production occured. Knowing these boundaries will help industrials to optimize tomato product formulation and storage conditions to limit mold and mycotoxin development during shelf-life.

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Modelling the Growth Rate of Listeria monocytogenes under the effects of growth factors and Carum copticum essential oil in TSB medium

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OBJECTIVES

Today, there is an increased concern about food safety due to increasing occurrence of new foodborne disease outbreaks caused by pathogenic microorganisms. Since there is significant consumer demand for foods which are antimicrobials such as essential oils are receiving a good deal of attention for a number of foodborne free from synthetic chemical preservatives with the perception of being natural, microorganisms reducing the need for antibiotics, improving shelf life extension technologies to eliminate pathogens, decreasing the development of antibiotic resistance by pathogenic microorganisms. Therefore, the present work was done to generate a model for predicting the growth rate of *Listeria monocytogenes* under the effects of *Carum copticum* essential oil, pH, temperature, and NaCl.

METHODS

Therefore in the present study combined effects of temperature (20–37 °C), pH value (4.5–7.5), concentration of sodium chloride (0–10.5% w/v) and concentration of *Carum copticum* (*Zenyan* in Persian) essential oil as a natural preservative (0-750 ppm), on the growth rate of *Listeria monocytogenes* were evaluated in five levels according to turbidity measurements in TSB broth model system. The growth curves generated within different conditions were fitted using Baranyi function. Subsequently the growth rate was modeled using quadratic equations of response surface (RS) model.

RESULTS

The established model was significant ($P < 0001$), and the RS model provided reliable estimates of the studied parameters with a bias factor between 1.004 and 1.038, and an accuracy factor between 1.167 and 1.220. SEP values ranged between 11.44 and 17.94, and RMSE values were within the range 0.009-0.019. All the four evaluation criteria were verified to be within acceptable range and the developed models provided an efficient method for predicting growth rate of *L. monocytogenes* as a function of controlling factors.

CONCLUSIONS AND IMPACT OF THE STUDY

The developed model was found to be significant and the predicted values were in good agreement with the experimental values. Moreover *Ca. copticum* essential oil indicated significant anti-listerial effects. Regarding all this, the established model, might be useful in predicting *L. monocytogenes* behavior in foods with a similar composition to the chemical and physical factors described in the present work; however they need validation in real food systems before being applied in practice.

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Quantifying the Bacillus weihenstephanensis and Bacillus licheniformis spore recovery considering the sporulation, the heat-treatment and the recovery conditions

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OBJECTIVE(S)

Spore-forming bacteria development in food is a major cause of food spoilage and food poisoning, leading to economical losses. Empirical models have been developed to predict spores in variable environments after heat-treatment (Mafart *et al.* 2010). The aim of this study is to understand and quantify *Bacillus weihenstephanensis* and *Bacillus licheniformis* spore recovery taking into account the properties induced by sporulation environments, the heat-treatments intensity and the recovery conditions. A predictive model, based on physiological parameters, will be designed to describe and quantify the spore recovery.

METHODS(S)

Bacillus licheniformis strain AD978 was isolated from raw dairy ingredients and was kindly provided by ADRIA Développement (Quimper, France) and *Bacillus weihenstephanensis* KBAB4 strain was kindly provided by the Institut National de la Recherche Agronomique (INRA, Avignon, France).. The sporulation was performed in sporulation mineral buffer (pH7.00) at two different temperatures: 12°C and 30°C for *Bacillus weihenstephanensis* and 45°C and 20°C for *Bacillus licheniformis.* Spores were treated at 95°C, 100°C and 105°C following the capillary method, in buffered peptoned water (pH 7.00). Then, a method of dilution-inclusion in the recovery media has been used for survivor counts (Baril *et al*, 2011). The spores were incubated in Brain Heart Agar plates at pH ranging from 4.50 to 8.00 and incubation temperature ranging from 4°C to 40°C for *Bacillus weihenstephanensis* and 15°C to 60°C for *Bacillus licheniformis.*

RESULTS

Inactivation kinetics have been obtained for each heat-treatment conditions and recovery temperature and pH. An optimal recovery has been observed at around 30°C for *Bacillus weihenstephanensis* and 45°C for *Bacillus licheniformis.* Moreover, the stronger are the conditions of heat-treatment, the stronger is the impact of the recovery medium. The spore heat-sensitivity *(z_T value)* was constant regardless to the recovery pH and temperature. Only the apparent heat-resistance (***8*** *value:* time for the first decimal reduction) was affected by the temperature and pH. Bigelow-like models, using *z'_T values*, have been previously used to fit the data but these models led to over-estimations and predict possible recovery at 15°C, while *Bacillus licheniformis* AD978 is not able to grow at this temperature. The model to be developed will be based on the growth physiological parameters (minimal, optimal and maximal recovery temperature and pH), which are often available in scientific literature and have a real physiological meaning. It shall also avoid over-estimation of the predicted heat-resistance out the range of temperature and pH allowing the growth.

CONCLUSIONS AND IMPACT OF THE STUDY

The new model would be useful to predict spore recovery after a heat treatment.The parameters used in this

model can be obtained by elicitations of expert opinion, allowing a possible extension to other bacterial species of concern for food safety and quality.

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Predicting the Growth Rate and Lag-Time of Staphylococcus aureus under the effects of Carum copticum essential oil, pH, temperature, and NaCl

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OBJECTIVES

During the recent decades, there has been a dramatic enhancement in research on the development of mathematical models. Growth predictive modeling has been so widespread that it is now one of the most rapidly advancing fields of study, which gives the ability to estimate the microbial growth in a swift and cost-effective way. Besides, there has been a global tendency towards using natural preservatives in food systems.

METHODS

Therefore in the present study combined effects of temperature (20–42 °C), pH value (4.5–8.5), concentration of sodium chloride (0–5%) and concentration of *Carum copticum* (*Zenyan* in Persian) essential oil as a natural preservative (0-750 ppm), on the growth parameters of *Staphylococcus aureus* were evaluated in five levels according to turbidity measurements in broth model system. The growth curves generated within different conditions were fitted using Baranyi function. Subsequently the two main growth parameters (maximum specific growth rate (GR), and lag-time (LT)) of the growth curves were modeled using quadratic equations of response surface (RS) model.

RESULTS

Mathematical evaluation demonstrated that the standard error of prediction (%SEP) and RMSE obtained by RS model were 68.285% and 0.030 for GR and 3.633% and 0.721 for LT for model establishing. Moreover *Zenyan* essential oil had significant antibacterial effect on *Staphylococcus aureus* (P value < 0.05).

CONCLUSIONS AND IMPACT OF THE STUDY

Overall according to the results elaborated RS model provided a useful and accurate method for predicting the growth parameters of *Staphylococcus aureus*, and could be applied to ensure food safety with respect to *Staphylococcus aureus* control.

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Inter-strain competition affects growth and detection of Listeria monocytogenes or Salmonella in foods by ISO methods

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OBJECTIVES

Classic pathogen detection methods in foods might not represent all strains initially present in samples, but those with competitive fitness, thereby introducing bias in detection. The objectives of this study were to investigate the competition among *L. monocytogenes* or *Salmonella enterica* strains during growth: (i) on laboratory media and foods and (ii) in selective enrichment.

METHODS

Growth of seven *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 1/2c, 4b) was tested in single and two-strain cultures (1:1 strain ratio). The competitive potential of seven *Salmonella enterica* strains (serovars Typhimurium, Reading, Agona, Emek, Putten) was also assessed in two and three-strain cultures. Strains were made resistant to different (rifampicin and streptomycin for *L. monocytogenes*, rifampicin, streptomycin and nalidixic acid for *Salmonella*) for their selective enumeration on Tryptic Soy Agar (TSA) supplemented with the respective antibiotics. Pathogens were inoculated (3 log CFU/ml or CFU/cm^2) in Tryptic Soy Broth (TSB), on TSA, and on vacuum packed sliced ham (5x5 cm^2) and stored at 10^{o}C. For the evaluation of inter-strain competition during enrichment, ISO 11290-1:1996/Amd 1:2004 and ISO 6579:2002 protocols were used, for detection of *L. monocytogenes* and *Salmonella* spp., respectively. Two enrichment steps including Half and Full Frazer enrichment broths for *L. monocytogenes* or Buffered Peptone Water and Rappaport Vasiliadis broths for *Salmonella* were performed. Both steps were followed by streaking on ALOA (*L. monocytogenes*) and XLD (*Salmonella*). Strain cultures were directly added in the enrichment broth or used to inoculate minced beef and sliced ham (3 log CFU/cm^2). The population of strains in enrichment broths was enumerated. 60-100 colonies were used to determine the relative percentage of each strain recovered on plates after streaking.

RESULTS

L. monocytogenes strains had similar ($p>0.05$) growth rates when cultured singly or in mixtures in TSB and TSA Conversely, growth on ham resulted in cases where a strain did not manage to increase in the presence of another strain. For *Salmonella*, the presence of competitors in laboratory media, had a significant ($p<0.05$) effect on the growth kinetics of strains. Depending on which strains were present in a mixture, growth rates of each individual strain could vary in some cases between 0.5 and 1.5 day^{-1}. Strains that were outgrown by others, did not manage to increase more than 7 log CFU/ml or cm^2 contrary to the 9 log CFU/ml or cm^2 final populations observed in single cultures indicating that maximum populations were also affected by inter-strain competition. During enrichment there were not significant differences among populations of different strains in enrichment (liquid) co-culture. However after streaking, *L. monocytogenes* 4b serotype outcompeted (80-100% of total colonies) the 1/2a serotype, regardless of the food matrix inoculated before enrichment. In BPW, the dominance (80-100%) of Reading and Putten serovars was reversed in the second enrichment in RVS (0-20% of total colonies).

CONCLUSIONS AND IMPACT OF THE STUDY

Understanding how strain competition of pathogens may affect their survival and growth may contribute to the improvement of detection methods and assist in outbreak investigations.

Modeling thermochemical effect of oregano essential oil on spoilage bacteria growth

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OBJECTIVE

The aim of this work was modeling the thermochemical effect (oregano essential oil (O.E.O.) and heat treatment) on the shelf life of processed mussels (*Perna perna*) in flexible packaging using Baranyi and Roberts model [1], to verify the influence of temperature on the growth kinetics of spoilage bacteria.

METHODS

Four treatments were performed for mussel (*Perna perna*) samples: A1 (control), A2 (heat treatment), A3 (O.E.O. without heat treatment), A4 (O.E.O. with heat treatment). The mussels were massaged with O.E.O., packaged in flexible pouches and subjected to pasteurization (80 ° C/10 minutes) and stored at 4, 10 and 15 ° C. The mussels shelf life was accompanied by microbial growth achieving scores of 10^7 CFU/g (lactic acid bacteria, total count of mesophilic, psychrotrophic and psychrophiles) and pH. The bacteria growth curves were modeled using Baranyi and Roberts model to different temperatures.

RESULTS

A decrease in pH was observed for all samples. The Baranyi and Roberts model showed good ability to describe the growth kinetics of spoilage bacteria. Thermochemically treated (O.E.O. and heat) increased the mussel shelf life. When the samples were stored at 4 °C, the shelf life was 21 days for control sample (A1) and for heat treated sample (A2). For samples with the addition of O.E.O. without heat treatment (A3), the shelf life was 31 days and when the O.E.O. was applied associated with heat treatment (A4), an increase on mussels shelf life (51 days) was observed. When the incubation temperature increased to 10 and 15 ºC, it has been observed a significant decrease in the shelf life of the mussels for all samples. Secondary models were applied and selected to describe the influence of temperature on microorganisms growth parameters.

CONCLUSIONS AND IMPACT OF THE STUDY

It can be concluded that the O.E.O. had antimicrobial effect on micro-organisms studied and that the thermochemical treatment prolong the mussels shelf life days from 21 (control) to 51 days, when stored at 4°C. The temperature effect is directly linked to the shelf life of food. Modeling the bacterial growth provide parameters (maximum growth rate, lag time) which describe the growth kinetics over time leading to a secondary modeling. In this study, the bacterial growth was successfully described by Baranyi and Roberts model. The thermochemical treatment, that combines use of essential oil with heat treatment, has proved to be a promising method for the preservation of mussels, because with the microbiological stability the product becomes appropriate to distribution processes, marketing and prolonged storage.

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Modeling inactivation of psychrotrophic yeasts strains in orange juice pH 4.0

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OBJECTIVE(S)

This research aimed to model the thermal inactivation of *Saccharomyces bayanus* and *Rhodoturula mucilagenosa* in orange juice, pH 4.0, single strength.

METHODS(S)

Test organisms, vegetative and spores cells suspension production

The spore suspension of *S bayanus* and *R.mucilagenosa* were produced from inoculum in PDA (25°C/5days), following which it was suspended in sterile water and stored at(2°C). 0.2mL aliquote of suspension was transferred to Roux bottles containing Yeast Malt Extract Agar (formulated, pH 5.5) added of manganese sulfate solution 5ppm; incubated at 25°C/30days. Spoliation was monitored until 90% of spore production. Spore collection was performed and centrifugations were conducted at 4°C/10min/3000rpm. Each suspension was standardized and transferred to a sterile bottle and stored at 4°C. Vegetative cells suspension of *Rmucilagenosa* was produced by inoculation in PDA (25°C/2days). Growth the yeast cells with mucilage were collected using sterile water and suspension was adjusted for $2.0xl0^{8}$CFU/mL, by Densimat (bioMérieux).

Heat medium and heating experiments

For heat resistance determination the capillary method was applied [1]. For this, 9mL of sterilized orange juice was added of lmL of vegetative/spore suspension and capillary tubes were filled with O.lmL. The temperatures applied were: for *S.bayanus:* 48, 50, 55 and 60°C and for *R.mucilagenosa:* 50, 55 and 60°C. The subculture was done in formulated Tetraciclin Glucose Yeast Extract Agar, added with lmg/mL of tetraciclin. Incubation was conducted for 5 days at 28°C, for both yeasts.

Treatment of thermal resistance data

Using obtained data (log of survivors x time) at each constant temperature, for each microorganism was constructed a survivor curve adjusting the data by GInaFiT program [2], for kinetic parameters estimation, using two models: Weibull [3]- equation 1 and log linear inactivation [4].

$$LOG10(N) = LOG10(N0) - \left(\frac{t}{\delta}\right)^{p} \quad \text{Eq. 1}$$

Where: 5 [time unit] is a scale parameter defined as the time for the first decimal reduction if p=l, and *p* [-] is a shape parameter. *Yoxp>f* convex curves are obtained, and for/?<7, concave curves;

$$N = No*exp(-krm\hat{}t) \quad \text{Eq.2}$$

Where: No/N - initial/final population, kmax - inactivation parameter, t – time

RESULTS

Saccharomyces bayanus

Inactivation for *S.bayanus* in orange juice wasn't linear for all tested temperatures. Data of survivors were adjusted by Weibull model with high R^2 values: at 48°C - $D^*_{48}{}_{o}{}_{C}$=2.09min, R^2=0.835, p=0.55 (Fig. la); at 50°C - D^*_{50}°c=0.87min, R^2=0.9594, p=0.56 (Fig.lb) and 55°C - D^*_{55}°c=7.11s, R^2=0.9594, p=0.95 (Fig.1c). At 60°C, no growth was recovered. Z value, obtained by linear regression was 5.67°C with R^2=0.9995.

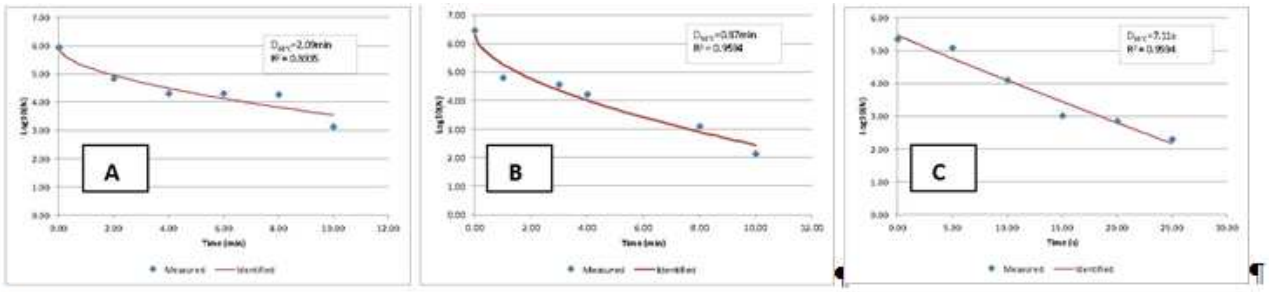

Figure 1. Thermal inactivation of *S.baynus* in orange juice pH 4.0 at 48, 50 and 55°C.

Rhodoturula mucilagenosa

For *R.mucilagenosa*, inactivation was linear for all tested temperatures for both cases. For vegetative cells, inactivation parameters were: at 55°C - $D_{55°C}$=4.87min, R^2=0.988; at 57°C - $D_{57°C}$=0.97min, R^2=0.999 and at 60°C – $D_{60°C}$=0.37min, R^2=0.998. Z value, obtained by linear regression was 4.6°C with R^2=0.9342. For spores of *R.mucilagenosa*, inactivation parameters were: at 55°C -$D_{55°C}$=3.92min, R^2=0.9972; at 57°C - $D_{57°C}$=1.20min, R^2=0.9968 and at 60°C – $D_{60°C}$=0.45min, R^2=0.9921. Was observed that at low temperature, 55°C, vegetative cells with mucilage was more heat resistant than spores; however, when temperature was increased, spores showed higher heat resistance. Z value, obtained by linear regression, was 5.45°C with R^2=0.972.

Comparing D values of *S.bayanus* and *R.mucilagenosa*, it is possible to observe that *R.mucilagenosa* was more heat tolerant than *S.baynus*, since at 60°C it was necessary 0.37 or 0.45min for a decimal reduction of vegetative cells or spores, respectively; and *S.baynus* didn't survivor at this temperature.

CONCLUSIONS AND IMPACT OF THE STUDY

Both yeast are heat sensible and don't survive common pasteurization treatment. If these microorganisms are found in the final product, they may be result on pos process contamination. The presence of mucilage in *Rhodoturula* doesn't increased the heat resistance as initially expected.

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Validating growth models under dynamic temperature profiles simulating temperature abuse seen in power failures using Salmonella spp. in raw ground beef

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OBJECTIVE(S)

Temperature is a primary factor in controlling the growth of microorganisms in food. When food is transported at ambient temperatures for extended periods of time, or when power is lost due to natural disasters; foodborne pathogens can multiply. Current US Food and Drug Administration (FDA) Model Food Code guidelines state that food can be kept out of temperature control for up to 4 hours or up to 6 hours if the food product starts at an initial 41°F (5°C) temperature does not exceed 70°F (21°C). This project validates existing ComBase computer models for *Salmonella* spp. growth under changing temperature conditions modeling scenarios that would exceed current FDA codes using raw ground beef as model system.

METHODS(S)

The growth of a cocktail of *Salmonella* spp. isolated from different meat products (*Salmonella* Copenhagen, *Salmonella* Montevideo, *Salmonella* Typhimurium, *Salmonella* Saintpaul, and *Salmonella* Heidelberg) was measured in ground beef. Inoculated ground beef samples were held in a programmable water bath at 5°C and subjected to linear temperature changes to different final temperatures over different lengths of time, and then cooled back to 5°C. The temperature profiles represent food being brought out of proper refrigeration, warming, and then cooling on linear gradients. A total of 9 different conditions were studied. Maximum temperatures reached were 16, 27, or 37°C, and temperature rises took place over 4, 6, and 8 hours with varying cooling times for each.

RESULTS

Our experiments show that when maximum temperatures were lower (16°C or 27°C), there was generally good agreement between the ComBase models and experiments. For example, when temperature rises to 16°C or 27°C took place over 8 h, experimental data were within 0.13-log CFU of model predictions. Furthermore, when the rise was to 16°C, no statistically significant increase in *Salmonella* concentration was observed (in agreement with model predictions). When maximum temperatures were closer to the optimum growth temperature for *Salmonella* (37°C), predictive models were fail-safe, predicting a 4-log increase, where only a 2-log increase was actually observed. Predicted lag times generally agreed with experimental data in all temperature profiles tested. In many of the conditions tested, a majority of growth happened during the 'cooling phase' of the experiment, after the temperature started the initial decline, but could still permit growth.

CONCLUSIONS AND IMPACT OF THE STUDY

This research gives insights to the validity of current available predictive models with regards to predictions under changing temperature conditions that are relevant to power loss due to natural disasters or to time periods where foods are held out of temperature control. It appears that faster cooling times help most to limit the growth of microorganisms, such that it may prove safer to rapidly cool foods (e.g. in a freezer) after extended temperature abuse. This validation data should prove extremely useful to consumers, restaurateurs that may transport food in unrefrigerated vehicles and to supermarkets and restaurants facing a power loss.

These finding may also be useful to those seeking to improve the science base of the FDA Model Food Code.

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Modeling Lactobacillus plantarum growth curves obtained using quantitative PCR (qPCR)

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OBJECTIVES

The need to ensure microbiological safety and quality of food has stimulated the application of predictive microbiology for modeling pathogenic and spoilage microorganisms growth in food. Thus, the behaviour of microorganisms can be quantitatively described with the aid of mathematical models that allow the description of their behaviour as a function of environmental parameters. The study of the shelf life of a food should be done taking into consideration their natural microflora. The lactic acid bacteria (LAB) such as *Lactobacillus plantarum,* are important agents of spoilage of chilled and vacuum packed meat products. Upon this, the aim of this study was to compare the growth curves obtained from viable cell count and quantitative PCR (qPCR), through modeling of the growth of the *L. plantarum* in its optimum growth temperature of 30 °C.

METHODS

The growth of pure cultures of *L. plantarum* was analysed in MRS Broth (Agar Man, Rogosa & Sharpe) at 30°C. The initial pH of the experiments was approximately 6.0. The flasks were incubated and the growth curves were monitored using viable cell counts and qPCR methods, until the stationary phase. The primary models fitted to the growth curves for the parameters obtained were: Gompertz and Baranyi-Roberts model. Growth curves were fitted using Matlab 7 *software.* To choose the model that best describes the primary growth, we determined the statistical contents: correlation coefficient (R^2), mean square error (MSE), bias factor and accuracy factor.

RESULTS

Tests conducted with *L. plantarum* in MRS Broth utilizing qPCR were compared with the method of viable cell count on MRS Agar. The primary model Baranyi-Roberts was used to describe the growth curves of *L. plantarum* tested at the optimal temperature (30 °C) and growth parameters were: duration of the lag phase (***k*** = 0.27 h), maximum specific growth rate (**H** = 0.652 h^1) and logarithmic increase in population (A = 14.17). The results obtained by qPCR were not statistically different than those obtained by plate count showing a satisfactory relationship between the two methods.

CONCLUSIONS AND IMPACT OF THE STUDY

This study demonstrated that qPCR is a promising alternative for the quantification of *L. plantarum,* since the statistical indexes obtained with qPCR did not present a significant difference between the results obtained with the traditional method of counting. Furthermore, qPCR for quantification of spoilage microorganisms in foods offers many advantages over other techniques, such as versatility, speed and sensitivity, making it a promising tool for improving safety and quality of food products. In order to simulate real conditions in animal foods, which may be damaged by LAB, there is a need to study the growth curves for mixed cultures of strains containing the LAB generally considered natural in meat and meat products. Through this study, the tool of qPCR can be an excellent alternative for the quantification of mixed culture

microorganisms that presents the same genre, allowing the construction of growth curves of each strain from mixed culture participant individually.

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Effect of the high pressure processing on Listeria spp. inactivation on smoked cod: Modelling the effect of processing parameters

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OBJECTIVES

Vacuum-packed cold-smoked fish products are considered as potential high-risk food for *Listeria monocytogenes*. The mild temperatures (<30 ºC) and low salt concentrations (<5%, water phase salt) used in the cold-smoked processing of fish are not sufficient to inactivate *L. monocytogenes*. In the last years, the application of high pressure processing (HPP) on fish products has been implemented by the food industry to control *L. monocytogenes*. In the present work, survival of non-pathogenic *L. innocua* as a surrogate for *L. monocytogenes* has been studied. The aim of the study was to quantify and model the effect of HPP on inactivation kinetics of *Listeria innocua* inoculated on smoked cod.

MATERIALS AND METHODS

Sliced smoked cod samples (*Gadus morhua*) with a mean weight of 1250± 100 g were obtained by a local manufacturer (Spain). Samples of smoked cod inoculated with *Listeria innocua* (*ca.* 10^7 CFU/g) were treated at different pressure and time conditions (200–600 MPa; 1.2 - 15.0 min). The high pressure treatments were carried out at AZTI-Tecnalia, using a hydrostatic pressurization unit (Wave 55HT, Hiperbaric, Burgos, Spain). After treatment, *Listeria* spp. was enumerated on Listeria selective agar base (CM0856 Oxoid ,Ltd., Basingstoke, UK) incubated at 37ºC for, at least, 48 h. Inactivation data (Log CFU/g *vs.* time) for each pressure level were fitted using different primary models, including the Log-linear, the Log-linear with tail and the Weibull models (Geeraerd et al. 2005). The effect of pressure (MPa) on the key kinetic parameters (kmax) of the selected primary inactivation model (Log-linear with tail) was described by simple secondary model. Model predictions were compared with the inactivation rates obtained from independent experiments with smoked cod treated by HHP. Comparisons between our model and the model developed for inactivation of *L. monocytogenes* by HPP on RTE cooked meat products (Hereu et al. 2012) were also carried out by estimating the bias and discrepancy percentages.

RESULTS

HHP process at 500 MPa for 15 min, and 600 MPa for 3, 5, 8 and 15 min, on smoked cod achieved a total inactivation of *L. innocua* on smoked cod. On the other hand, it is worth noting that HHP at 200 MPa had not significant bacterial inactivation effect at any treatment time. The HHP-inactivation kinetics of *L. innocua* inoculated on sliced smoked cod was appropriately described by a Log-linear model with tail. Model predictions were compared with the inactivation rates obtained from independent experiments. In this case the discrepancy percent was 27.69% and mostly due to bias percent of -20.72%. These results indicate that predicted inactivation rates obtained by our model were smaller than observed inactivation rates, resulting in safe-fail predictions. Moreover, our predictions for inactivation of *L. innocua* were compared with the model developed for inactivation of *L. monocytogenes* by HPP on RTE cooked meat products (Hereu et al. 2012). Discrepancy and per cent bias were 26.57 % and 20.03%, respectively, indicates that predictions of these two models are quite similar taking into account that models are based in different matrices (smoked fish and RTE cooked meat products) and different *Listeria* species.

CONCLUSIONS AND IMPACT OF THE STUDY

We have assessed that pressures of 500 MPa and 600 MPa are effective for inactivation of *L. innocua* in smoked cod. This work shows the Log-linear with tail model as the most appropriate primary model to fit HHP inactivation kinetics of *L. innocua* on smoked cod. HPP might be used as a hurdle technology approach against *Listeria* spp., increasing the safety of these products. In addition, the results of this work may help smoked cod processors to select optimum processing conditions of HHP technology.

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APPLICATION OF PREDICTIVE MICROBIOLOGY FOR FOOD INDUSTRY AND RISK MANAGERS

Oral conferences

An App for That: Future Tools for Predictive Microbiology, Risk Assessment and Decision Support for Food Business Operators

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The past few decades have seen significant advances in the application of mathematical models and information technology to support decision-making related to food safety and quality. These advances in understanding and predictive capacity range in scope from the properties of individual micro-organisms up to the performance of entire food production, marketing and related systems at the national and international levels. At the same time, the development of tools available to support food business operators may be significantly behind what would be possible with the right level of investment focussed on decision support. This presentation will review some significant advances, and reflect on parallel advances that are occurring in related public health oriented disciplines. Looking forward, it will describe some ideas and opportunities for the predictive microbiological community in providing an improved mathematical, scientific and technological basis for improved food safety decision-making.

Greg Paoli serves as Principal Risk Scientist and COO at Risk Sciences International, a consulting firm specializing in risk assessment, management and communication in the field of public health, safety and risk-based decision-support. He has experience in diverse risk domains including toxicological, microbiological, and nutritional hazards, air and water quality, climate change impacts, and engineering devices, as well as risk assessment for natural and man-made disasters. He specializes in probabilistic risk assessment methods, uncertainty analysis, the development of risk-based decision-support tools and comparative risk assessment. Greg has served on a number of expert committees devoted to the risk sciences. He was a member of the U.S. National Research Council committee that issued the 2009 report, Science and Decisions: Advancing Risk Assessment, also known as the Silver Book. He serves on the Canadian Standards Association Technical Committee on Risk Management, overseeing the Canadian adoption of ISO 31000. He serves on a US NRC Standing Committee on the Use of Public Health Data at the U.S. Food Safety and Inspection Service, and has served on several expert committees convened by the World Health Organization. Greg completed a term as Councilor of the Society for Risk Analysis (SRA) and is a member of the Editorial Board of Risk Analysis. Recently, Greg was awarded the Sigma Xi – SRA Distinguished Lecturer Award. Greg holds a Master's Degree in Systems Design Engineering from the University of Waterloo.

Food Safety Risk Ranking of E. coli O157:H7 and Salmonella in Fresh Fruits and vegetables: Structured Risk Ranking Approach using FDA iRisk Tool.

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OBJECTIVE

Given the complex nature of the food supply and the wide array of foodborne risks, setting priorities and appropriately allocating resources is an ongoing challenge for all governments. Evidence-based approach is now required by regulatory bodies to evaluate and compare food safety risks posed by microbial and chemical hazards. *Escherichia coli* O157:H7 and *Salmonella enterica* are among the most frequently associated with foodborne diseases resulting from the consumption of fresh produce (Doyle and Erikson, 2008; Sivapalasingam *et al.*, 2004). Although several risk ranking tools and methods already exist (Chen *et al.* 2013), their readiness to inform real time food safety decision making remains a limitation. The Canadian Food Inspection Agency is developing a new approach for food safety risk ranking using a Web-based database and quantitative risk assessment tool (FDA iRisk) to evaluate and compare the health impact of microbial and chemical food hazards. This presentation describes a case study on *Salmonella* and *E. coli* O157:H7 in various fresh fruits and vegetables in which the FDA-iRisk model framework and templates were applied to define risk scenarios and estimate the health burden. The CFIA's approach also explores ways to integrated non-health related parameters such as market impact, social sensitivity and consumers' acceptance and perception.

METHODS

Risk scenarios were defined using *Salmonella* and *E. coli* O157:H7 and the following fresh fruit and vegetable commodities: leafy herbs, leafy greens, cantaloupe, bean sprouts, and tomatoes. The model inputs included technical information on the hazards (concentration, prevalence), the food process model (impact of food production, processing, and handling), and the exposed population groups and resulting health end points. For both pathogens, the Beta-poisson dose-response model has been selected to describe the relationship between the dose ingested and the probability of illness (Teunis et al., 2008). Serving sizes and number of eating occasions were derived from the Canadian national single day food consumption report (PHAC, 2012) and computations were generated through Monte Carlo simulation with the ultimate risk output being the annual DALYs (Disability Adjusted Life Years).

RESULTS

The case study demonstrates the ability of the FDA iRisk tool to group fresh fruits and vegetables associated with *Salmonella* and *E. coli* O157:H7 in an ordered manner based on the total risk metric. From library constituted in the FDA iRisk, up to four risk scenarios were developed for each food and hazard pair, taking into consideration total population as well as children under 5 years, children from 5 to 10 years, children of more than 10 years and adults under 60 years, and senior people (more than 60 year old). The model results include final pathogen concentration and prevalence, total number of illnesses, mean risk of illness, number of eating occasions, and the annual DALYs. Options for using the health impact metric (DALYs) in a multi-factorial framework with other parameters such as market impact, social sensitivity, and consumers' perception is also presented.

CONCLUSIONS AND IMPACT OF THE STUDY

Food safety risks can be evaluated readily and ranked based on their public health impact using a structured gathering and analyzing technical and scientific information. This case study demonstrated the capability and flexibility of the FDA iRisk tool to support this approach. This will have a considerable impact on the governments' needs to build food safety systems that are science and risk-based.

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Quantitative study of the effect of processing of fresh-cut lettuce on the distribution of Escherichia coli O157:H7 and Salmonella: Application to design sampling plans.

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INTRODUCTION

Vegetables can become contaminated along the food chain with enteric pathogenic bacteria such as *Escherichia coli* O157:H7 and *Salmonella* spp. Recent several outbreaks and studies suggest that contamination by these pathogens is sporadic (Critzer & Doyle, 2010) and no data are available on how these microorganisms are distributed in leafy green produces. Knowing enteric pathogen distributions in leafy green produces could be crucial to assess potentials sampling plans and elucidate microbiological criteria.

OBJECTIVES

To study the distribution of enteric pathogenic bacteria in a batch of fresh-cut lettuce by simulating a commercial process at laboratory scale and based on results to derive adequate sampling plans for pathogen detection.

METHODS

An industrial fresh-cut lettuce processing was simulated at laboratory scale. Different initial contamination levels were assayed (6, 5, 4, 2 and 1 log cfu/g) in order to study the different contamination distributions obtained in the final product after processing. A head of lettuce was artificially contaminated by the pathogen and then was introduced in a 30-kg batch to be submitted to the typical steps of a fresh-cut vegetable process: cutting, mixing, washing, centrifuging and packaging. The entire batch was packaged into 50-g bags and then all bags and the whole content was analyzed for quantification and investigation of each pathogen. Counts and presence/absence data were analyzed and processed to assess the type of distribution (i.e. homogenous, overdispersion, etc.). For that, different probability distributions were fitted in order to account for pathogen distribution after processing, and stochastic simulations were performed.

RESULTS

Outcomes demonstrated a homogeneous distribution of contamination through bags for all assayed inoculum levels. Even at low concentration, most samples were contaminated, and censored data were obtained. These results would suggest, as expected, that the processing gives rise to a homogeneous distribution of microorganisms, which could be also evidenced by standard deviation (SD) values, always below 1 and most cases, below 0.5. In addition, a negative relationship between coefficient of variation (CV) and initial inoculum were observed, and a linear model was proposed as a first approach to explaining this mathematical relationship. The regression analysis concluded that bacteria distribution could be adequately described by a log-normal distribution at the different inoculation levels. Also, further analysis suggested that bacteria distribution followed a Poisson process. Simulation performed, using different probability distributions, and assuming a Poisson process, confirmed that the Poisson lognormal distribution would be the best choice to represent sampling of fresh-cut lettuce. Therefore, this approach was later used to assess potential sampling plans, based on a linearity assumption between CV and initial contamination, and extrapolating results to extreme low initial concentrations (<<-2.5 log cfu/g). When the initial mean

concentration was low, i.e. -6-(-4), a probability of rejection of around 99 % was obtained by analyzing 20 samples.

This number decreased as the mean contamination increased, and when the lot mostly contained contaminated samples, with only 5 samples, the probability of finding a contaminated sample was 99 %. The use and extrapolation of the found relationship between CV and mean could affect results and therefore, a simulation of contamination at pilot/industrial scale would be needed to enable lower levels and elucidate in a broader range the relationship between initial levels and distribution of pathogen in the final product (i.e. mean and SD or CV).

CONCLUSIONS AND IMPACT OF THE STUDY

Results enabled to propose suitable probability distributions for describing distribution of *E. coli* O157H7 and *Salmonella;* and to derive sampling plans based on generated probabilistic models. In addition, these probability models could be used in future quantitative risk assessment studies.

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A quantitative exposure assessment of Bacillus cereus in cooked chilled foods

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OBJECTIVE(S)

The objective of the exposure assessment was to determine which steps in the production and distribution of cooked chilled foods (CCF) had the most significant effect on the exposure of consumers to *Bacillus cereus* from these products. The exposure assessment was built to address three different questions: (i) The regulatory authority wanted to know if there was a safety risk, how they should direct their inspections (where to sample) and what was the importance of consumer behaviour. (ii) The manufacturers wanted to know whether they could reduce the pasteurisation treatment without compromising food safety. (iii) And both wanted to have a better understanding on how Food Safety Objective and Performance Objective (PO) could be translated into Process criteria (PrC) at the operational level.

METHODS(S)

An exposure assessment model was developed for the processing and shelf life of CCF, using the Modular Process Risk Model (MPRM) approach (*1*). The model consisted of nine modules: raw material reception, product formulation and assembling, mild cooking, intermediate storage, partitioning, mixing, packaging, pasteurisation and distribution into the supply-chain up to consumer plate. For each model a combination was made of literature and self-gathered data (*2*), own models (*3,4*) and expert input from four cooked chilled food manufacturers. The model was implemented in @Risk (Palisade) software and run using Monte Carlo simulation (10^6 iterations). In the absence of a dose-response relation for *B.cereus*, a 10^5 CFU/g acceptability threshold was used. One baseline simulation and 13 scenarios were compared.

RESULTS

The results of the baseline scenario showed that three modules were crucial in terms of making products exceed the threshold: Raw material contamination, recontamination during packaging and storage in the consumer fridge. Scenario-analysis showed that reducing raw material contamination (by 1 log) and reducing the consumption of products after their 'use by' date were the most effective strategies to reduce the number of products that exceeded the threshold. Increased hygiene during packaging was less effective, but decreased hygiene significantly increases the exposure.

Results also showed a counterintuitive effect of purchasing frequency; consumers with a high frequency of purchase (e.g. multiple cooked chilled foods per week) had a lower relative probability of exposure to *B. cereus* from CCF than consumer with a low frequency of purchase (e.g. 2-3 a month). This was a direct consequence of the fact that frequent consumers store the product less long (*4*), thus reducing the probability that *B. cereus* grows.

In addition to scenario analysis, a series of Iso-exposure curves were plotted. They showed a trade off between pH, a_w, pasteurisation (T and time) and shelf life. Reducing pasteurisation without increasing the exposure was possible when the pH was reduced or the shelf life shortened. Finally specifications were

drafted for two PO's: raw material contamination and *B. cereus* concentration prior to pasteurisation. For the latter PO a set of PrC was determined resulting in the same PO. A set of iso-PO plots was made for the time-temperature of intermediate storage.

CONCLUSIONS AND IMPACT OF THE STUDY

The exposure assessment determined the critical points in the CCF production. It indicated that vigilant supplier selection, a rigorous cleaning and disinfection plan and consumer education, were the three main options for reducing the consumer exposure to *B. cereus*. It revealed that reducing pasteurisation was possible if other hurdles for bacterial growth were also considered (mainly pH and chilled temperature).

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A Decision Support Tool for Multi-Quality Forward and Inverse Commercial Food Problems

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OBJECTIVES

Mathematical models are becoming ever more important to predict and enhance the (nutritional) quality and shelf-life of foods. Existing software programs for (microbial) food quality modeling are deficient because 1) they cannot provide all the model parameters needed for the myriad of new products and processes, and 2) the model parameters provided in the software are almost always based on laboratory constant-temperature simulations and not on dynamic commercial processing conditions. Therefore, the objectives for this work were to develop a user-friendly software tool that will: a) perform both forward (prediction) and inverse (parameter estimation) problems for a number of different primary-secondary models, b) identify model parameters under static and dynamic conditions, and c) give statistical indices to discriminate among models. To show the usefulness of this tool, a set of dynamic microbial growth data were chosen as an example.

METHODS

A prototype software was developed as a turnkey, simple graphical user interface (GUI) with click-buttons in MATLAB executable code. Previously published data (Patil, Valdramidis, Tiwari, Cullen, & Bourke, 2011) describing the growth of *S. cerevisiae* in apple juice with square-wave variation of temperature between 4 and 16°C over 24 days were used. The primary model chosen was the differential form of the Baranyi growth model (Baranyi & Roberts, 1994). The secondary model chosen was the square root model relating the maximum growth rate with the temperature (Ratkowsky, Olley, Mcmeekin, & Ball, 1982). The tool permits uploading microbial and time-temperature data, selecting between a number of models, entering initial guesses of the parameters, and generating the initial scaled sensitivity plots. The five parameters ($y0$=log$N$(0) [log(cfu/mL)], $ymax$= logN_{max} [log(cfu/mL)], h_0 [-], b [day^{-1}°C^{-2}], and T_{min} [^{o}C]) were estimated sequentially from the dynamic data (inverse problem). A table of results was generated from the « Tabulated Results » option. Lastly, the forward problem was performed by predicting the growth curve of *S. cerevisiae* for another dynamic temperature profile representative of a chilled distribution warehouse.

RESULTS

The « Estimate Parameters » selected option generated four plots on one page: 1. Experimental and predicted log growth versus time; 2. Final scaled sensitivity coefficients; 3. Residual plot; and 4. Sequential estimation of all five parameters. The RMSE = 0.21 log(cfu/mL) out of a 4.5 log total span. Scaled sensitivity coefficients were uncorrelated and large (favorable results), except that for *h0*. Residuals were reasonably uncorrelated. The relative errors of all five parameters were under 5.4%, with low correlation coefficients. Sequential estimates came to a constant about halfway through the experiment, showing that the model was appropriate for the data.

CONCLUSIONS AND IMPACT OF THE STUDY

The novel contributions of this work are:

1. The non-expert user can estimate parameters directly from the dynamic data;
2. The parameters are estimated from data collected on the user's microorganism and food of interest, and are not taken from a database;
3. The program determines whether the parameters can be estimated and which parameters will be most accurate, by using scaled sensitivity coefficients;
4. The changes in the parameters are shown as the experiment progresses;
5. The program can handle both the inverse and forward problems;
6. The tool is compiled and deployed as an executable file which does not require a license of MATLAB by the users.

If data can be collected directly from a commercial, dynamic process, this software can be used to estimate parameters (the inverse problem), and then to predict growth levels (the forward problem) for other realistic, "what-if" conditions. Processors will find this software useful to analyze and design their processes to improve quality and ensure safety.

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Modeling number of bacteria per unit vs. bacterial concentration in quantitative risk assessment: impact on risk estimates and modeling complexity

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OBJECTIVE

Quantitative microbial risk assessment (QMRA) models typically evaluate the dynamics of bacterial populations in food across the food-production chain. Usually, a "farm-to-fork" model is split into basic processes such as growth, inactivation, partitioning, mixing, cross-contamination, etc. (Nauta, 2008). Because of the diversity of situations that may be modeled, there is no standardized modeling approach. Some published QMRAs model changes in bacterial concentration, others consider the number of bacteria per unit and still others consider both in the model depending on the basic processes. The objective of this study was to evaluate the impact of these modeling approaches on risk estimates as well as model complexity.

METHODS

As a case study, we developed a risk assessment model of Enterohaemorrhagic *E. coli* (EHEC) contamination in fresh-cut lettuce. Various fresh-cut lettuce food-production chains were evaluated, including various processing steps: inactivation of EHEC on lettuce in the field and during washing, cross-contamination during washing, mixing of lettuce leaves in a bag, EHEC growth during transport, salad partitioning into serving sizes. For each basic process, relevant parameters were extracted from the published literature.

The models were developed using three approaches. The first simulated the bacterial population concentration (log10 cfu/g) for all steps of the process. At each step, the bacterial population was then characterized by some real number. A second approach considered the number of bacteria per unit at each step and used only natural numbers ($n > 0$). A third approach simultaneously considered the prevalence of contaminated units and the number of bacteria in positive units (i.e., natural number > 0). The dose response model was adapted to consider the average EHEC concentration or the number of EHEC cells in the serving at time of consumption.

The three approaches were compared in terms of complexity, expected number of cases, variance in the number of cases, and model convergence.

RESULTS

For most simple food-production chains, the expected number of cases for a given process appeared comparable when modeling concentration vs. number of bacteria. Working on concentrations had the advantage of greater modeling simplicity using readily available software packages (such as Excel add-ins). Moreover, model convergence was obtained more rapidly. However, this approach considerably under-estimated the variability in risk estimates. Cross-contamination was not easily modeled using this framework, and some additional assumptions were needed. In the second approach, modeling the number of bacteria without explicitly considering prevalence led to convergence problems when the bacterial concentration was low. Modeling both the number of bacteria and the prevalence enabled a full evaluation of risk variability and readily allowed modeling of cross-contamination, but led to a slower convergence than

the approach based on the concentration alone.

CONCLUSIONS AND IMPACT OF THE STUDY

The choice of whether to model bacterial concentrations or the number of bacteria in a QMRA has clear consequences on modeling results and model convergence. A better understanding of those consequences will enable selection of the most adequate framework.

Modeling prevalence and the number of bacteria in positive units might allow modeling of more process steps typical in the farm-to-fork food chain than modeling a composite concentration distribution encompassing prevalence, and might enable better modeling of complex processes such as cross-contamination.

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Risk assessment model for Shiga-toxin-producing Escherichia coli in ground beef in France: efficiency of different strategies of intervention and sampling beef trim or ground beef

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OBJECTIVE(S)

In this study we developed a quantitative microbial risk assessment (QMRA) model to assess the public health risks associated with the consumption of ground beef contaminated with Shiga-toxin-producing *Escherichia coli*, including the five Main Pathogenic Serotypes of STEC (MPS-STEC) that have been identified in Europe (O157:H7, O26:H11, O103:H2, O111:H8 and O145:H28). The model was then used to evaluate the relative efficiency of various interventions at different steps of the food chain. Moreover, the model was used to evaluate the efficiency of different strategies of sampling.

METHODS(S)

The model considers typical ground beef plants producing batches of 10 000 to 50 000 of ground beef patties from carcasses slaughtered in France. The QMRA model is based on previous QMRA studies (Nauta et al., 2001 and Cassin et al., 1998). The model combines four modules: farm module, slaughterhouse module, ground beef production and retailing module and consumer module. The outcome of the first module is the probability distribution of the prevalence of shedding animals entering French abattoirs and the concentration of STEC in their feces. The prevalence of MPS-STEC was estimated using data from a recent survey conducted in France (Auvray et al., 2012). The second, third and four modules consider the basic processes – growth, inactivation, partioning, mixing, contamination and cross-contamination – as described by Nauta (2001). The model incorporates recent data on ground beef handling, preparation and consumption collected in France. Particularly, data on thermodynamics during cooking and strains heat resistance were generated for different types of ground beef cooking. The risk associated to the consumption of contaminated ground beefs was then estimated using a modified exponential dose-response model with age as a covariate.

Three type of interventions were implemented in the model: primary preventive measures against STEC contamination of meat, interventions that are expected to decrease prevalence or concentration of STEC in feces, secondary prevention measures that include slaughtering and ground beef production process hygiene measures, and tertiary prevention measures which are intervention taken during ground beef handling, preparation and cooking just before consumption.

Finally, the model includes different strategies of sampling beef trim or ground beef using a cost benefit analysis.

RESULTS

The combination of primary and secondary prevention measures showed the highest impact on the Public health risks associated to the consumption of contaminated ground beef by one of the five Main Pathogenic Serotypes of STEC identified in Europe can be reduced (Risk reductions ranged from to 75% to 99.9%). Tertiary preventive measures have the potential to bring additional risk reductions but with higher uncertainty (30% to 99.99%) due to the variability of consumer compliance with cooking recommendations. Sampling of beef trim coupled with rapid and specific testing for STEC could be used as an additional control measure. The predicted risk reduction varied from 15% to 96% when sampling is applied without other interventions and 1% to 96% when applied in combination to other interventions. The proportions of

positive batches varied from 5% to 50%.

CONCLUSIONS AND IMPACT OF THE STUDY

This study showed clearly how microbial risk assessment models can be a useful tool to compare the relative efficiencies of different intervention strategies. Combination of the current possible primary and secondary prevention measures do not guaranty STEC-free product but in some circumstances reduces significantly the public health risks. Sampling beef trim and ground beef for STEC should not be abandoned.

Our study demonstrates that batch acceptance sampling is a tool that should be combined with the different type of prevention measures and calibrated in regard to the current food safety achievement and the food safety objective.

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Risk-based control and management of Listeria monocytogenes and Salmonella spp. in short- and long-maturated Italian fermented sausages

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OBJECTIVES

Although fermented foods are generally recognized as safe, some notable outbreaks of foodborne illness associated with fermented foods have occurred (Adams and Mitchell, 2002). The extent to which fermented foods are safe and how fermentation processes should be conducted to achieve a required level of safety are critical questions. Depending on manufacturing conditions, foodborne pathogens may survive at the end of the process. Therefore, the objective of this study was to evaluate the risk that *Listeria monocytogenes* and *Salmonella* spp. pose to the consumer at the time of consumption of Cacciatore and Felino sausages and to demonstrate how the results can be used to manage the risks by setting Performance Objectives (PO) and/or Performance Criteria (PC) during manufacturing of these products to achieve a suggested Food Safety Objective (FSO).

METHODS

Cacciatore and Felino batter was inoculated with a cocktail of *L. monocytogenes* or *Salmonella* spp. strains (*ca*. 10^5-10^6 cfu/g) and their survival was monitored at specific time points during sausages manufacturing. For each pathogen and sausage, four (4) different batches (replicates) were prepared at different times. Cacciatore has short maturation time (20 days) whereas Felino ripening lasts up to 40 days (long maturation time). Various models were tested and the best model was selected, based on several statistical indices to calculate the parameters of interest (inactivation rate, k_{max} in day^{-1}; and time needed for 1 log reduction of the pathogen, D-value in days). Model fitting was performed using GraphPad Prism 5. Significant factors (pH and a_w) for *L. monocytogenes* and *Salmonella* spp. inactivation were assessed by multiple regression using SPSS v15.1. The results were used to provide examples for risk management of fermented sausages. FSO for the considered pathogens was determined according to Perni et al. (2009).

RESULTS

The inactivation of *L. monocytogenes* and *Salmonella* spp. in both sausages was log-linear. Inactivation of *L. monocytogenes* during manufacturing of Cacciatore (k_{max}=0.04, D-value=52) and Felino (k_{max}=0.02, D-value=111) was negligible (0.4 log cfu/g). On the other hand, *Salmonella* spp. displayed faster inactivation (k_{max}=0.13 and 0.09, D-value=18 and 24, total inactivation=1.1 and 1.6 log cfu/g for Cacciatore and Felino, respectively). Multiple regression showed that a_w was a significant parameter ($P<0.05$) for *Salmonella* spp. inactivation in both products, explaining 60-70% of the variance observed in the data. For *L. monocytogenes*, a_w was significant ($P=0.002$) during its inactivation in Felino (40% of explained variance) and pH (0.003) in Cacciatore (50% of explained variance). A part (10-15%) of the remaining unexplained variance was attributed to the applied temperature. The ICMSF equation (2002) was used to make implications about the PO and PC for controlling pathogens in both sausages.

CONCLUSIONS AND IMPACT OF THE STUDY

Salmonella spp. proved to be more sensitive than *L. monocytogenes*. Both pathogens, however, survived relatively well as result of the conditions (pH, a_w and fermentation temperature) prevailing during manufacturing of the sausages. A_w proved to be a key factor. Quantitative analysis of the data originating from challenge tests may provide critical information on which combinations of the process parameters would potentially lead to better control of the pathogens. Moreover, the analysis clearly identified factors that need to be validated experimentally, i.e. Felino characteristics lie within the range that may support growth of *L. monocytogenes* according to EC regulation 2073/2005 and its amendment 1441/2007. Although, raw materials quality constitutes a critical control point, the safety of these products is almost totally dependent on proper fermentation-ripening (killing step). Pathogens should be below certain levels at specific process stages (initial contamination level and inactivation during fermentation-ripening) to meet a FSO.

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Predictive model for Vibrio parahaemolyticus numbers in Pacific oysters (Crassostrea gigas): attempting to validate under New Zealand conditions

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OBJECTIVE(S)

The primary aim of this work was to evaluate the numbers of naturally occurring total and pathogenic *Vibrio parahaemolyticus* (a causative agent for gastroenteritis) in response to storage at different environmental temperatures and to compare the results with those predicted by the Oyster Refrigeration Index software developed by the University of Tasmania Food Safety Centre (Tamplin et al., 2012).

METHODS(S)

Five hundred and fifty Pacific oysters were harvested from the same site located in the North Island of New Zealand during summer. The water temperature and salinity were measured as 22°C and 35 ppt at the time of harvest. Samples were shipped cold (ca 10°C) to the laboratory in polystyrene boxes. On arrival, the oysters were distributed in open plastic trays and incubated at different temperatures: 5, 10, 15, 20, 25 and 30°C. Three sets of 12 oysters were immediately assessed to estimate the initial count of microorganisms (T0). The remaining oysters were sampled at least at four time points and subjected to microbiological analysis (Kaysner et al., 2004). Multiplex real-time PCR analyses were conducted to confirm *V. parahaemolyticus* and identify pathogenic strains (*tdh* and *trh*) (Nordstrom et al., 2007). The total and pathogenic population of *V. parahaemolyticus* was estimated using the Most Probable number (MPN) (Blodgett et al., 2006). The data were analysed using mathematical Dynamic Modelling (DMFit - ComBase) developed by the Institute of Food Research, UK (Baranyi et al., 1994) and compared to those predicted by the Oyster Refrigeration Index.

RESULTS

The initial population count of total *V. parahaemolyticus* averaged 1.6 x 10^2 MPN/g, while pathogenic strains, *trh* and *tdh*-*V. parahaemolyticus*, were not detected. *V. parahaemolyticus* was inactivated at a rate of –0.0072 log MPN/h when oysters were incubated at 5°C. A growth rate of 0.0052 log MPN/h was observed at 10°C and was similar for samples stored at 15°C. The growth rates increased logarithmically with increasing temperatures (0.0490, 0.0363 and 0.0399 log MPN/h at 20, 25 and 30°C respectively). The highest population counts were observed at 20 and 25°C (4.2 log MPN/g). Pathogenic *tdh*-*V. parahaemolyticus* were observed occasionally but did not provide enough data to be analysed. Pathogenic *trh*-*V. parahaemolyticus* was not observed initially (T0), but were found positive in all subsequent sampling points at all temperatures. Moreover, relatively high levels of the latter were observed at 10°C and 15°C (11.37 MPN/g and 24.16 MPN/g respectively). Growth rates for *trh*-*V.parahaemolyticus* at 10 and 30°C were 0.009 and 0.002 log MPN/h, respectively. Our study showed similar pattern for total *V. parahaemolyticus* in Pacific oysters harvested in Tasmania, except for samples stored at 10°C. Growth at this temperature was mainly observed at the last sampling point (196 h) and could reflect the population variability within samples (Fernandez-Piquer et al., 2011). The Tasmanian predictive model developed using samples inoculated in the laboratory over-estimated *V. parahaemolyticus* numbers at higher temperatures (25 and 30°C), as well as at 5°C, while it underestimated numbers at 10°C. Predicted and observed data were in agreement at 15 and 20°C.

CONCLUSIONS AND IMPACT OF THE STUDY

This preliminary data shows that the Tasmanian predictive model currently available might cause unnecessary costs due to overestimation of counts at some temperatures, but may also represent a hazard to public health by under-estimating counts under conditions of transport and storage.

We believe that the predictive model developed for *V. parahaemolyticus* in Pacific oysters would benefit from a wider set of data to minimize the effects of intrinsic variability between samples and to identify any other factors that may affect the growth of microorganisms.

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Risk assessment of Clostridium perfringens in Cornish pasties in the UK

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OBJECTIVE

The objective of this study was to develop a stochastic risk assessment model for *C. perfringens* that could easily be adapted for different types of meat pies and pasties using product specific data on the prevalence of the pathogen in the raw ingredients and the time and temperature of cooling and storage steps. The model is described for Cornish pasties, a Protected Geographical Indication (PGI) meat pie consumed in large quantities in the UK.

METHODS(S)

To estimate the risk associated with the consumption of pasties the risk assessment model was built from the time point the pasties exit the oven after baking until the moment of consumption. The risk of acquiring a *C. perfringens* toxico-infection was estimated taking into account growth in contaminated ingredients as a result of inappropriate cooling at room temperature for pasties of different sizes in combination with variation in consumer practices regarding cooling.

RESULTS

The model predicted a mean of 549 (95% CI: $2.3 \cdot 10^{-3}$ - $3.5 \cdot 10^{3}$) cases of *C. perfringens* toxico-infections per million inhabitants each year due to the consumption of Cornish pasties, whose individual contribution varied considerably depending on their size, with mean estimates ranging from 7.5 to 148 cases per million inhabitants.

CONCLUSIONS AND IMPACT OF THE STUDY

Major factors influencing this estimate and possible targets for interventions were the time to consumption and the concentration in contaminated ingredients, the impact of which in reducing the risk was tested in different intervention scenarios. The results of the model together with possible interventions to reduce the risk are used as a platform to discuss the setting of the Appropriate Level of Protection (ALOP) and Food Safety Objective (FSO) targets for this particular pathogen-product combination.

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Modelling microbial behaviour in foods to perform safety assessment. Industry perspective.

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Historically, Food Business Operators (FBOs) have relied on microbiological criteria of end product to perform the verification of their food management systems. The recently introduced microbial risk management metrics such as Food Safety Objectives, Performance Objectives and Performance Criterion (FSO, PO, PC) have been proposed for the establishment of a more direct relationship with public health outcomes based on the SPS 1995 defined Acceptable Level of Protection (ALOP).

Better knowledge of the food product process is now expected both by consumers and food safety authorities, most particularly vs. growth potential / survival rate of hygienic and safety microbial species of concern. *Listeria monocytogenes* in Ready To Eat (RTE) Foods, *Cronobacter spp.* in Powder Infant Formula (PIF): risk assessment studies from the FAO/WHO JEMRA and Guidelines from Codex Alimentarius provide the framework to FBOs to provide documentation of the safety of their own process.

Numerous Microbial Risk Assessment Guidelines are now available (US FDA, Codex, EPA, FAO/WHO, …) with quantitative software online or through specific collaborations / consultations. Tools for prediction of microbial behaviour in foods have to provide a challengeable output in term of validation and transparency to help FBOs performing the safety assessment of their processes. While internal tools can be developed to provide a fine tuned answer, use of external peer reviewed models are also needed to be reviewed by external stakeholders.

While the accessibility of the approach has been improving in the recent years, FBOs did not communicate precisely on their numerous expectations on the topic. Nowadays, most of the available published data addresses Food Safety Issues, while FBOs are also (if not mostly) interested in assessing the impact of the process on the quality of the end product and validate shelf life, most particularly in case of extended durability of one food product. As the awareness of the potential of this approach grows among FBOs, there will be place for innovation and practical applications in the near future (if not already in place though not always applied correctly).

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He has collaborated within ILSI Europe task forces on risk analysis in food microbiology and emerging microbiological issues and within the standing committee on microbiological hygiene of the International Dairy Federation. He has been member of the Sym'Previus project since its earliest year while working for Soparind Bongrain and Danone.

APPLICATION OF PREDICTIVE MICROBIOLOGY FOR FOOD INDUSTRY AND RISK MANAGERS

Posters

Decision Support Tools to ensure safe, tasty and nutritious Advanced Ready-To-Eat foods for healthy and vulnerable Consumers (STARTEC)

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Food business operators must make daily decisions about food safety and quality, often based on limited scientific data, or full knowledge of the consequences of deviations for the consumer, due to limited capacity to carry out analyses and risk assessments.

The purpose is to present the STARTEC project, and how industrial production conditions can be taken into account in design of experiments and development of models for decision support.

This three-year EC-funded project (STARTEC) will develop the necessary tools based on scientific evidence and predictive and probabilistic models to enable food operators to estimate the safety and quality level in their products (ready-to-eat foods) if alternative ingredients, process and storage conditions are used. Pathogens being studied include *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Bacillus cereus*, Verotoxinproducing *E.coli* (VTEC) and Clostridium spp, as well as Staphylococcus toxins. High-pressure treatment, dielectric heating, biopreservation and packing technologies are being investigated, along with food quality. The project focuses in particular on vulnerable consumers where increased quality and safety levels are needed, e.g. patients at nursing homes and hospitals, or elderly and sick people living at home. The project started in 2012 and involves eight participants across Europe, of which five are SMEs. Producers of convenience and RTE products are actively involved, as well as experts in food microbiology, food chemistry, food process technology, information technology and modelling, laboratory analytical methods, cost-benefit analysis and risk assessment. The decision-making tool will enable the SME operator to quantify and manage spoilage and pathogen risks in a way that is not currently possible. A cost-benefit module in the tool will allow food producers to compare quality, safety and costs of their actions, or consequences of abuse conditions along the food supply chain. The prototype will be tested and validated during the project.

During the first year of STARTEC, we have observed large scale production of ready-to-eat products like deli salads and convenience products like full meals for microwave heat treatment in three companies. The purpose has been to map the characteristics in large scale production which are likely to influence the safety and quality of the products. Beside the risk of unintended conditions like crosscontamination between product, delays in production leading to periods with abuse temperature, improper cleaning, etc, normal conditions in industrial production like inhomogeneity of products, slow heat transfer within the production batch, only light heat treatment of some components in products in order to ensure the sensory quality, etc all have an impact on the food safety and quality. The observations have been used to develop downscaled model systems for experimental studies with pathogens in food in the lab where large scale production characteristics have been taken into account. The relevance of these downscaled systems are that realistic industry production conditions are more correctly estimated than in experiments in ideal model systems.

Some results from studies using these model systems will be shown.

"STARTEC is funded by the EC (Grant No: 289262) under 'FP7-KBBE.2011.2.4-01- Safety and quality of ready-to-eat foods'. Coordinator: Dr. Taran Skjerdal, Norwegian Veterinary Institute, Oslo, Norway. www.startec-eu.info"

Estimation of the public health impact of setting a new target for the reduction of Salmonella in turkeys in the European Union

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OBJECTIVES

Regulation (EC) No 584 sets a transitional target for reduction being less than 1 % of flocks remaining positive for *Salmonella* Enteritidis and/or *Salmonella* Typhimurium by the end of 2012 both in flocks of breeding and fattening turkeys. For assessing the beneficial public health impact of a new target for reduction of *Salmonella* in fattening turkeys beyond 2012, the Scientific Panel on Biological Hazards (BIOHAZ) of EFSA received a request from the European Commission. The Panel was also asked to indicate and rank the *Salmonella* serovars with public health significance in turkeys.

METHODS

A 'Turkey Target *Salmonella* Attribution Model' (TT-SAM) based on a microbial-subtyping approach[1] was applied. The model considered the quantitative contribution and relevance of different *Salmonella* serovars found in turkeys to human salmonellosis. The frame of the model is similar to the one that was developed in a former EFSA assessment of targets for *Salmonella* in broilers (*Gallus gallus*)[2]. TT-SAM included 25 MSs, four animal-food sources (i.e. turkeys, broilers, laying hens and pigs) and 23 *Salmonella* serovars. TT-SAM employed (i) prevalence and serovar distribution data from the 2010 EU statutory monitoring (turkeys, broilers and laying hens) and 2006 EU-wide baseline survey (slaughter pigs); (ii) data on incidence and serovar distribution of reported cases of human salmonellosis, and (iii) food availability data, including amounts traded between MS. MS-specific underreporting factors of human salmonellosis were applied. Seven scenarios where *Salmonella* prevalence in turkey flocks were changed were assessed and the results compared to the results of the baseline TT-SAM model.

RESULTS

Based on the TT-SAM model[3,4], in 2010, there were approximately 5.4 (95 % CI: 3.0-9.5) million true cases of human salmonellosis in the EU. It estimated that around 2.6 % (95 % CI: 1.2-5.2), 10.6 %, 17.0 % and 56.8 % of the human salmonellosis cases could be attributed to reservoirs relating to turkeys, broilers, laying hens (eggs) and pigs, respectively. The top-six serovars of fattening turkeys that contributed most to human cases were *S.* Enteritidis, *S.* Kentucky, *S.* Typhimurium, *S.* Newport, *S.* Virchow and *S.* Saintpaul. However, when comparing the risk of turkey meat with the other three sources weighted by the amount of food available for consumption, the risk of infection was highest when consuming table eggs closely followed by pig meat, whereas the risks associated with broiler and turkey meat was approximately two-fold lower. Comparing the situation in 2010 with a theoretical combined prevalence for *S.* Enteritidis and *S.* Typhimurium of 1 % (i.e. the transitional target), the expected reduction in the percentage of turkey-associated salmonellosis cases was small (0.4 %). In 2010, all MSs except one had already met the transitional target. However, when adjusting the combined prevalence of all serovars to 1 %, a large reduction (83.2 %) in the percentage of turkey-associated salmonellosis cases was expected, equivalent to 2.2

% of all human salmonellosis cases. The individual MS contribution to the estimated reductions varied greatly. Uncertainty and data limitations have been considered, including recommendations on how these could be overcome.

CONCLUSIONS AND IMPACT OF THE STUDY

Based on the outcome of this assessment, the transitional *Salmonella* target in flocks of fattening turkeys has been confirmed[5].

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Monte Carlo Simulation applied to Listeria spp. exposure assessment in a new vegetables mixed beverage

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Vegetable and fruit juices industry is gaining attention due to healthy and ready-to-eat character of new formulated beverages. However, some microbial pathogens are frequently contaminating raw vegetables and could represent a potential risk on minimally processed vegetable based products; specifically, *Listeria monocytogenes* which has been described as food borne pathogen of concern due to its psychrotolerant character and serious listeriosis sympthoms. In this sense, the present study assesses the thermal resistance (50-65 °C) of a *Listeria monocytgenes* surrogate (*L. innocua* (910 CECT)) in the new vegetables formulated beverage, order to guarantee the accomplishment of food safety objectives (5D). A new tomato, Italian pepper, celery, cucumber, carrot, onion, mixed beverage was formulated and supplemented with olive oil, lemon juice and basil powder (0.03% (w/v)). Three levels of pH 5.20, 4.75 and 4.25, were studied in the novel beverage by means variation of lemon juice addition 0.5, 1, and 2 % (v/v), respectively. Inactivation kinetics experimentally obtained results were mathematically fitted to Gompertz equation. According to the obtained results, 5 log cycles reduction in *L. innocua* initial load was achieved after 65°C-30s, 65°C-50 s, and 65°C-100 s (pH=5.20) in vegetable beverages with pH values 4.25, 4.75 and 5.20, respectively. Thermal inactivation kinetic parameters of the Gompertz equation were obtained and accuracy of the model fit was assessed with adjusted $-R^2$ [0.85-0.99] and RMSE [0.10-0.22] coefficient values. Maximum death rate (μ_{max}, log(cfu/ml)/min) was significantly affected ($p < 0.05$) by both treatment temperature (50-65ºC) and beverage pH, with maximum μ_{max} value (15.68±0.85 log(cfu/ml)/min) at 65 °C in beverage with 4.25 pH value. From a microbiological safety point of view, optimization of present process design and product formulation requires the assessment of process/product changes on estimated microbial risk. In this sense, Monte Carlo simulation was used by means of a stochastic modellization approach to determine consumers exposure levels derived from the consumption of the studied vegetable mixed beverage in a service size of 100 ml. Initial contamination level, kinetic Gompertz parameters, and beverage pH were considered as inputs in the stochastic modellization and were fitted to probability distributions defining different scenarios under study. Monte Carlo simulation was run and *L. innocua* final log counts were probabilistically obtained at different run scenarios. The final contamination results obtained by simulation of vegetable beverage single service of 100ml consumption reveals that exposure risk after 65 °C-100 s beverage processing was reduced by means 10^6 factor when formulation changes increasing lemon juice addition from 0.5 (pH =5.20) to 2 % (pH = 4.25).

Applicability: Present study points out the impact of process and product formulation factors on listeriosis risk by exposure to *L. monocytogenes* surrogate after consumption of a new thermally processed vegetable beverage.

Keywords: vegetable beverage, exposure assessment, Monte Carlo simulation, thermal treatment, listeria monocytogenes

A Model and Software for Microbial Risk Assessment

Cian O' Mahony[1]

1. *Creme Global*

OBJECTIVE(S)

To develop a model and software for estimating consumer exposure to foodborne pathogens.
Quantitative microbial risk assessment involves linking exposure assessment to dose-response models to characterise consumer risk. In order to accurately assess consumer exposure to foodborne pathogens, the concentration of pathogen in food at the time of consumption must be known, as must the quantity of food consumed. The former is dependent on the initial concentration of pathogen and environmental conditions in the supply chain, while the latter is dependent on the dietary habits of a population of consumers. It is possible to integrate measured pathogen concentrations in foods and/or predictive microbiology with dietary exposure into one food safety system for microbial risk assessment.

METHODS(S)

Experimental data from the ComBase database was used to derive model parameters for the Baranyi Roberts microbial growth model under a range of experimental conditions for different pathogen/commodity pairs. To test the system the model was linked to foods consumed in the US NHANES dietary survey to assess pathogen exposure based on actual consumption levels, using a probabilistic exposure model and an appropriate dose response model. For a given pathogen/commodity pair, variable conditions in the supply chain were simulated using random sampling from parameter ranges derived from environmental conditions to examine the effect on population exposure and illness. Multiple scenarios can be simulated simultaneously by assigning relative weights or likelihoods to different storage conditions.

RESULTS

A population of 100,000 consumers and the incidence of foodborne disease can be simulated using the model for a given food pathogen (based on a sample population of 8,406 from NHANES 2010). Using a high performance cloud based system the entire calculation can be performed within 30 minutes. Scenario and sensitivity analysis can be used to assess the effect of various control strategies on the incidence of disease, e.g. lowering temperature during distribution or reducing time spent in retail storage.

CONCLUSIONS AND IMPACT OF THE STUDY

A model for assessing consumer risk under real and variable conditions in the supply chain has been developed, that can assess multiple and variable conditions in the supply chain simultaneously. This allows for a more realistic and holistic risk assessment that can be used to determine which control measures have the greatest impact on minimising the occurrence of foodborne illness.

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Bayesian Estimation of Norovirus Risk in a Ready-to-Eat Food Production Line

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OBJECTIVES

Noroviruses are the most common cause of non-bacterial gastroenteritis in humans. In this study, a Bayesian evidence synthesis model was developed in order to estimate norovirus prevalence in a ready-to-eat food and to evaluate main contamination sources in a food processing environment. Based on background information and other studies, three contamination sources were included in the simplified model: (1) Food can become contaminated during the process with direct contact by an infected food handler. (2) Surface (production line) contamination may lead to food contamination. (3) If its raw-material is contaminated with viruses, the end-product will also be contaminated. The data used in the model consist of information on contamination sources and test results of the final product. However, only limited and sparse data are available about model parameters.

METHODS

The first part of the model estimates norovirus prevalence in the three contamination sources. The food will become contaminated if viruses are transmitted to it from the contamination source. The transfer rates were estimated based on experimental data and literature information. The proportion of contaminated products caused by each source was estimated by combining the information on the prevalence and the transfer rate of viruses. Finally, the Bayesian evidence synthesis model combines information on the different contamination sources as well as data from output variable, as a day-to-day process in a Markovian discrete time model, conditional to the total set of data. Sample data from the food production process output, the ready-to-eat product, also allows 'backwards' learning about the parameters of the different contamination sources.

RESULTS

As a result of the model, we get joint posterior distribution for the parameters of interest. In preliminary results, the proportion of the contaminated ready-to-eat foods with at least one virus caused by food handlers or production surface was estimated to be at its highest in March (0.64%) and at its lowest in September (0.12%). The estimated number for the operation days with at least one infected food handler or contaminated surface was 1.2 (CI 95%: 0 - 11).The computations of the model were performed using WinBUGS software.

CONCLUSIONS AND IMPACT OF THE STUDY

The model developed in this study offers an effective way to combine multiple sources of quantitative evidence in order to produce numerical information on the contamination risk of ready-to-eat foods. However, more data should be collected for more accurate results on all contamination sources. Due to relatively rare infection events which nevertheless can have large effects, Monte Carlo computations are challenging. In addition, this model can be used as a tool in order to do scenario calculations with artificial data.

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Predicting the effect of washing and chilling on microbiological quality of poultry carcasses by applying meta-analysis

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OBJECTIVES

Meta-analysis is a statistical method analyzing a relatively large amount of data from different individual studies to produce a more precise estimate of a particular intervention/treatment or to identify sources of variation. It is widely used in medicine and social sciences but not in food microbiology (Gonzales-Barron and Butler, 2011). The objective of this study was to advance the methods used in predictive microbiology by presenting a statistical approach that has a limited application in the field. Specifically, meta-analysis technique was used to predict/investigate 1) if washing and chilling interventions have an impact on poultry carcasses microbiological quality, 2) if yes, the summary effect of these interventions and 3) the sources of heterogeneity between studies.

METHODS

Literature search was performed to find studies dealing with poultry carcasses microbiological quality. All published papers, reporting total number of carcasses analyzed, mean and standard deviation of total viable counts (TVC) before and after the poultry carcasses washing and/or chilling, were included. TVC was the outcome because more data were available for this parameter, leading to increased statistical power of the performed meta-analysis. A random-effects model and Hedges's g (hg) as summary effect size were used due to different experimental designs between studies (microbiological analysis: excision, swab or whole carcass rinse-WCR; washing: spray or shower; and chilling: water or air). Sub-group analysis was carried out to investigate sources of heterogeneity and the obtained hg values were compared by three different statistical tests. The Mix 2.0 software was employed to run meta-analysis.

RESULTS

The heterogeneity statistics for washing ($Q=251.93$, $P<0.001$; $I^2=94.05\%$; and $\tau^2=1.42$) and chilling ($Q=10890.28$, $P<0.001$; $I^2=99.82\%$; and $\tau^2=1.20$) revealed that correctly a random-effects model was used due to presence of heterogeneity between studies. Forest plots indicated a significant ($P<0.001$) beneficial (TVC reduction) effect of washing (hg=-1.88) and chilling (hg=-1.78) on poultry carcasses microbiological quality (combined summary effect). Since effect size varies substantially from one study to the next our focus should shift from the summary effect to the dispersion itself and try to explain this variability. Type of microbiological analysis (destructive, excision; and non-destructive, swab and WCR) and equipment for washing (spray or shower) and chilling (water or air) were used as study-level covariates to perform sub-group analysis. Regarding equipment, significant hg values ($P<0.01$) were obtained (TVC reduction) but their statistical comparison revealed significant difference ($P<0.01$) between water- (hg=-2.93) and air-chilling (hg=-0.90) but not ($P>0.05$) between spray- (hg=-1.51) and shower-washing (hg=-3.11). Regarding microbiological analysis, significant hg values ($P<0.001$) were also obtained (TVC reduction) for washing (destructive, hg=-2.68; and non- destructive, hg=-0.99) and chilling (only for non-destructive, hg=-3.33; destructive, hg=-0.18 with $P=0.60$). Interestingly, significant differences ($P<0.01$) between destructive and non-destructive methods were found in both, washing and chilling. Dispersion (I^2) was still high £75%) meaning that other study covariates, e.g. sampling site of the carcass, TVC contamination level at the

slaughterhouse, country or year may have an impact as well.

CONCLUSIONS AND IMPACT OF THE STUDY

Both processes improve microbiological quality of poultry carcasses by reducing TVC but the measured reduction is dependent not only on the washing or chilling equipment but also on the microbiological analysis and potentially to other factors. Excision and swab/WCR have an effect on the measured microbiological quality resulting in different recoveries for TVC (higher for excision). Water-chilling was more effective than air-chilling, whereas spray- and shower-washing seem to be equally effective.

Therefore, it is not correct to report a combined summary effect for the two types of washing or chilling which could indicate that washing or chilling is, on average, moderately effective since this is true for neither group (microbiological analysis and equipment) and misrepresents the core finding.

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Meta-analysis and Bayesian modeling in quantitative microbiological risk assessments -Specific application to Salmonella spp. prevalence after chilling of pork carcasses

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OBJECTIVES

Meta-analysis synthesizes data from various individual studies producing a combined estimate of the question under concern with increased statistical power. Meta-analysis has not been widely used in quantitative microbiological risk assessments (QMRA) (Gonzales-Barron et al., 2009). Meta-analysis and Bayesian modeling allow better estimation of the intervention/treatment true effect. The first leads to a combined summary effect, while the second combines prior knowledge with experimental data at hand (meta-analysis results) and thus estimates are obtained with better handling of uncertainty, a critical parameter in QMRA studies. Therefore, the objective of this study was to demonstrate how meta-analysis and Bayesian modeling can be considered in QMRA studies estimating 1) the impact of chilling intervention on *Salmonella* spp. prevalence during pork carcasses production, and 2) the combined summary effect.

METHODS

The study was based on the work of Gonzales-Barron et al. (2008). The main differences of the present study are summarized as follows:

1) use of a random-effects model accounting for between-study variability,
2) inclusion of additional studies found in the literature mainly performed after 2008,
3) treatment of each group within a single study as it were a separate study (independent subgroups),
4) inclusion of prediction intervals reflecting the uncertainty of the combined summary effect and
5) modeling the combined effect size using Bayesian analysis.

All published papers, reporting binary/dichotomous data for *Salmonella* spp. before and after chilling of pork carcasses (total number of carcasses analyzed, positive or negative to *Salmonella* spp. presence carcasses), were included. Relative risk (rr) was used as summary effect size. Bayesian modeling and meta-analysis were performed using WinBUGS v1.4 (Spiegelhalter et al., 2003) and Mix 2.0, respectively.

RESULTS

A fixed-effects model was run but the obtained heterogeneity statistics ($Q=44.34$, $P=0.005$; $I^2=48.13\%$; and $T^2=0.22$) uncovered between-studies variation and thus a random-effects model was considered since the effect size was not robust enough across the studies domain. Synthesis results ($rr=0.40$, $P<0.001$) showed that chilling process may have positive effect (reduction of *Salmonella* spp. prevalence) yet prediction intervals, reflecting uncertainty, identified potential pathogen increase after chilling. Dispersion indicated that some of the observed variability can be explained by carcass sampling alone (48%) but the remaining is owned to other factors, e.g. carcass sampling site, type of chilling equipment, etc. The effect size, however, varies modestly ($25\%<I^2<50\%$), hence the summary effect is valid but the true effect in any given study could be somewhat lower or higher than this value. In random-effects model, it is usually assumed that true effects are normally distributed. Consequently, a Bayesian model was constructed to make inferences about summary effect size [$rr=0.31$ or $\ln(rr)=-1.18$] and predictive distribution [$N(-1.18,0.65)$] for the effect of

chilling in a new trial. This value is lower than the one found from the meta-analysis. The Normal distribution allowed the effect size to vary from -2.62 to 0.26. The values above 0 indicate a probability (3.5%) for *Salmonella* spp. increase. This was not considered in Gonzales-Barron et al. (2008) paper, which holds when *Salmonella*-free herds are slaughtered immediately after the slaughter of *Salmonella*-positive herds (Botteldoorn et al., 2003; Bouvet et al., 2003).

CONCLUSIONS AND IMPACT OF THE STUDY

Meta-analysis, the product of synthesis of different studies, facilitates risk managers, policy and decision makers to have access in reliable and comprehensive information on the effect of specific interventions/treatments for controlling pathogens.
When meta-analysis is combined with advanced statistical techniques such as Bayesian modeling then the output distribution of the summary effect size of a(n) intervention/treatment is better estimated in terms of uncertainty and can indisputably be included in QMRA studies modeling the pathogen prevalence during the production of pork carcasses.

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Development of a decision support tool for corrective actions during storage of fermented sausages in case of pathogens survival during their production - Specific application to Listeria monocytogenes

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OBJECTIVES

Depending on fermentation and ripening conditions of sausages, *Listeria monocytogenes* may survive well during their production (Drosinos et al. 2006). Given the absence of an additional antimicrobial step after manufacturing, most often storage at high températures before distribution could be the only option and this may accelerate the inactivation of several pathogens including *L. monocytogenes* (Gounadaki et al. 2005). Therefore, the objective of this study was to compile the available data on *L. monocytogenes* survival during storage of vacuum-packaged fermented sausages at various températures in order to develop a generic model for decision-making purposes.

METHODS

Literature search was performed to identify ail the studies reporting quantitative data relative to *in situ* survival of différent *L. mono*cytogenes strains being in différent physiological states at the time of inoculation. A part of the studies were kept aside for validation purposes. Tool performance was assessed by the bias (B_f) and accuracy (A_f) factors. The log-linear (Bigelow and Esty, 1920) and two populations (Cerf, 1977) models were used to calculate the kinetic parameter of interest, i.e. inactivation rate (k_{max}). The 4D-value parameter was originated from the corresponding k_{max}. Linear régression was used to model log4D-value *vs.* température for determining z_{4D}-values. A secondary model was developed for modeling the effect of sausage water activity (a„) on z_{4D}- and V4D-values at 25°C. Multiple régression was employed to identify significant predictors of the secondary model. Model fitting évaluation was done by inspecting the R^2 and Root Mean Sum of Squared Error (RMSE) indices. Model fitting and parameters calculation were carried out using GInaFiT vl.6 (Geeraerd et al. 2005), linear régression using Excel and multiple régression using SPSS vl5.1.

RESULTS

Generally, the inactivation curve of *L. monocytogenes* appeared in two forms: the linear and biphasic. Because it was not possible to describe ail inactivation curves by a common model, the time needed for 4 logs réduction (4D-value) was used instead the classical D-value (time needed for 1 log réduction of the pathogen). Modeling log4D-value=/(T) showed good corrélation (R^2=0.76-0.99). The observed 4D-values were compared to predicted ones obtained by the Pathogen Modeling Program (PMP). The comparison revealed that 4D-values at 25°C agreed relatively well (Bf=1.01 and Af=1.40) and therefore this température was chosen as référence ($4D_{25}$-value). Multiple régression indicated that $4D_{25}$- and z_{4D}-values were dépendent only on a_w (P=0.007 and P=0.041 for $4D_{25}$- and z_{4D}-values, respectively) and not on pH (P=0.266 and P=0.769 for $4D_{25}$- and z_{4D}-values, respectively), explaining a signifïcant part of the variability observed in $4D_{25}$- (79.3%) and z_{4D}-values (92%). Therefore, only a_w was included as predictor variable in the secondary model. Finally, the décision support tool was successfully validated against the studies not initially used (B^l.08 and A^l.28).

CONCLUSIONS AND IMPACT OF THE STUDY

The developed $V4D_{25}$- and z_{4D}-value models can be used to predict the desired time-temperature combinations that lead to additional pathogen réduction. The décision support tool can predict the fate of *L. monocytogenes* at a spécifie storage température and based on this prédiction a décision could be made (corrective action) about the time needed to store the product before its distribution in order to achieve the additional desired pathogen inactivation.

It should be noted, however, that model applicability lies within the studies domain used to develop the décision support tool, i.e. vacuum-packaged fermented sausages with post-ripening pH from 4.5 to 5.0 and a_w from 0.82 to 0.92, and storage at températures from 4 to 30°C. Such tools can also be incorporated in HACCP studies of a food-producing company to assure food safety of its products.

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Establishment of Food Safety Objectives for Clostridium botulinum in meat-based products in order to set subsequently the thermal treatment which ensures product safety

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OBJECTIVE

The concept of ALOP (Appropriate Level Of Protection) and FSO (Food Safety Objective) have been suggested by respectively the World Trade Organization and the Codex Alimentarius to make food safety management tools more transparent and quantifiable. However, up to now the implementation of these concepts is difficult, due to a lack of agreement and practice regarding their use (Stringer 2005).
Our objective was to investigate how establishing a FSO for *Clostridium botulinum* in heat-treated meat-based product, in order to set subsequently appropriate thermal treatments in food process units. The two examples were chosen as follows. On one hand, products widely consumed with high outbreak incidence were selected; on the other hand products with low volume of consumption and low outbreak incidence were chosen. From these two groups, cook ham and canned foie gras were kept as case studies.

METHODS

This study is based on epidemiological data from French institute for public health surveillance (INVS) between 2001 and 2011 (11 years). The total number of *C. botulinum* outbreaks in France, the proportion of outbreaks related to cook ham and canned foie gras, an estimation of the non-reported cases, were collected.
The estimation of ALOP and FSO was based on the top-down approach described by Gkogka et al (2013). The hazard characterization and particularly the dose-response curve were derived from *C. botulinum* in honey outbreaks (Doyle 1989) and by seeking INVS expert information. The consumption pattern was given by the food manufactures.

RESULTS

The mean ALOP estimation was 0.23 and 0.012 cases/million inhabitant per year for cook ham and canned foie gras, respectively. Although those two values were significantly different, the FSO estimated for the two products were in the same order of magnitude (- 9 log cfu/g) due to significant difference in term of consumption pattern. The FSO values are in a range of FSO values reported for *C. botulinum* in shelf stable products (Anderson et al 2011).

CONCLUSIONS AND IMPACT OF THE STUDY

FSOs for C. botulinum in cook ham and canned foie gras were estimated. This result is an important step and may help to validate or revisit the heat-treatment settings of meat-based products potentially contaminated by Clostridium botulinum.

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Quantitative Exposure Assessment of Listeria monocytogenes in Ready-to-eat Salads

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OBJECTIVE

Microbial risk assessment (MRA) is a process used to evaluate the likelihood of adverse human health effects occurring after exposure to a pathogenic microorganism. *Listeria monocytogenes* is a ubiquitous, psychrotrophic pathogen that may contaminate different foods. Most human listeriosis cases appear to be caused by consumption of refrigerated ready-to-eat (RTE) foods that are contaminated with high levels of *L. monocytogenes*. Since the implement of Food Safety Law of the People's Republic of China in June 2009, China government has enforced the research of food risk analysis especially from the National Food Safety Risk Assessment Expert Committee of China founded in 2009. Among the previous researches, there was seldom focus on the risk of *L. monocytogenes* in RTE salads in China, especially the application of Quantitative MRA on *L. monocytogenes* in RTE salads. Therefore, a quantitative exposure assessment was conducted to model the risk of *L. monocytogenes* foodborne illness associated with the consumption of RTE salads in a city of China.

METHODS

This study used data from previous surveys on *L. monocytogenes* in RTE salads obtained in accordance to the National Standards of China with 100 typical samples to estimate the initial contamination level. And this initial level was described by a discrete distribution combining initial prevalence and concentration of *L. monocytogenes* in RTE salads. Meanwhile, the equation in the research of Jarvis (2000) was used in this study to estimate the "fail-safe" conditions of RTE salads, which means non-detectable levels of *L. monocytogenes* in positive RTE salads higher than the detection limit 10^2 CFU/g. Then, mathematical models from our previous study were used to test the growth kinetics of *L. monocytogenes* in RTE salads under different conditions from retail to home. The Logistic model and Ratkowski square-root model was used to describe the growth of *L. monocytogenes* in RTE salads by the effects of time and temperature, respectively. The parameters of time and temperature from retail to consumption of RTE salads was translated into the Normal and Pert distribution, respectively. The @risk software basing on Monte Carlo was applied for simulating the final probability of RTE salads containing more than 10^4 CFU/g *L. monocytogenes*, which suggested some potential risk for consumers.

RESULTS

Investigation revealed that 98 of the 100 batches of RTE salads contained *L. monocytogenes* at levels lower than 10^2 CFU/g. Based on Monte Carlo analysis using the @Risk software, different contamination levels were described using relative probability distribution. During the retail phase of RTE salads, the discrete distribution for the initial contamination level ranged from -4.09 to 3.97 lg CFU/g, with -0.94 log CFU/g as the average level. The probability of RTE salads being contaminated by *L. monocytogenes* at levels greater than 4 log CFU/g was 0.80% from the initial contamination. The results indicated that the risk caused by contaminated RTE salads with *L. monocytogenes* increased systematically and showed the highest risk at the end of retail storage due to the long holding time. The concentration of *L. monocytogenes* in RTE salads prior to public consumption was considered as the final contamination level. The probability distribution for the simulated contamination level of *L. monocytogenes* exhibited a minimum value of -3.09 log CFU/g (at the 5% level), a median value of 0.06 log CFU/g (at the 50% level), and a maximum value of 4.96 log

CFU/g (at the 95% level). Moreover, sensitivity analysis indicated that initial contamination level (r=0.95) was the main risk factor that could provide useful information to consumers and stakeholders.

CONCLUSIONS AND IMPACT OF THE STUDY

The application of quantitative exposure assessment of *L. monocytogenes* in RTE salads, also based on the establishment of predictive growth models, this study was focus on the possible hazard of *L. monocytogenes* in China.

The result of *L. monocytogenes* probability in RTE salads was fairly low. Combined with further dose–response relationship research data, the results of this study could be referenced for complete establishment of *L. monocytogenes* risk assessment. The results could be referenced to evaluate the risk of *L. monocytogenes* foodborne diseases associated with the consumption of RTE salads in China.

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Ranking risks posed by composite food products

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OBJECTIVES

EU legislation defines composite products as "*foodstuff intended for human consumption that contains both processed products of animal origin and products of plant origin and includes those where the processing of primary product is an integral part of the production of the final product*" (Decision 2007/275/EC). To develop harmonised risk-based public health rules for implementation during import of certain composite products, the European Commission asked the European Food Safety Authority (EFSA) to identify and profile the microbiological hazards for public health related to import of certain composite products (EFSA, 2012).

METHODS

A method based on two complementary approaches was developed to rank microbiological risks in composite products.

In the first approach, first the hazards were categorised in three categories (no growth needed for infection, growth needed for infection, growth needed for toxin production). For all three hazard categories a decision tree was developed to evaluate their evolution in food during processing and following steps up to consumption to qualitatively estimate the risk associated to composite products. Composite product/hazard combinations were ranked as posing *low risk*, *moderate risk* or *qualified presumption of risk* (i.e. if present in the product, the pathogens considered have the potential to cause disease via its consumption).

In the second approach, risks were evaluated based on the analysis of European Union data on the past occurrence of hazards in composite products and foodborne outbreaks. The available data and their importance were assessed and scored by experts.

RESULTS

Application of the first approach

Soup stocks, flavourings, meat extracts, meat concentrates, and sterilised heat-treated foods without possible recontamination were considered to be in general of *low risk*. Bread, low moisture biscuits/cakes/chocolate, sweets, dry pasta and noodles, food supplements, and unfilled gelatine capsules in general do not permit growth of pathogens. These products are therefore of *low risk* with regard to hazards that need to grow in food to cause illness. They may pose *moderate risk* or *qualified presumption of risk* with regard to hazards that do not need to grow in food to cause illness. The other composite products considered, such as high moisture biscuits/cakes/chocolate/confectionery, fresh pasta and noodles, and olives with fish, may pose *moderate risk* or *qualified presumption of risk*.

Application of the second approach

Very high importance of association resulted for *Salmonella* spp. and cakes, biscuits, bread and bakery products. High importance was identified for the association of *Staphylococcus aureus* with cakes and for the association of *Salmonella* spp. with chocolate/confectionery and with pasta/noodles.

CONCLUSIONS AND IMPACT OF THE STUDY

The two approaches should be integrated based on the assumption that whenever the first approach leads to a low risk, this is due to the intrinsic composition or processing of the food, independently of the information available from the second approach. Whenever the application of the first approach leads to a likely risk for a given food, the risk can be further qualified with the results from the second approach.

Among the foods resulting in *moderate risk* or *qualified presumption of risk* according to the first approach, combinations of hazards and categories of composite products of highest concern would be the ones for which a high to very high importance of association was identified by the second approach, i.e. *Salmonella* spp. in biscuits, bread, cakes, chocolate, some types of confectionery and sweets, pasta and noodles, and *S. aureus* (enterotoxins) in some types of cakes.

The method proposed is considered suitable for application with success to other types of food, and is proposed as a tool for risk managers to rank foods based on their potential food safety risks.

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Estimation of Performance Objectives for Campylobacter in broiler carcasses taking into account impact of selected factors on pathogen prevalence and counts

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OBJECTIVE(S)

Consumption of contaminated broiler meat accounted for 20 to 30% of campylobacteriosis human cases in EU in 2011, (EFSA, 2013). The establishment of risk based performance objectives (POs) at slaughtering is recognized as an efficient strategy for its eradication. The current paper provides a strategy to elucidate a potential Food Safety Objective (FSO) and subsequently derive possible POs for *Campylobacter* according to the different scenarios of prevalence and concentration as assessed in the EFSA monitoring study performed in 2008 in the European Union.

METHODS

The dataset used is part of the EU-wide baseline survey of *Campylobacter* in broiler meat carried out in the EU in 2008 (EFSA, 2010). A deterministic approach was selected to derive a potential FSO (Pérez-Rodríguez et al., 2007), which was used as benchmark to elucidate POs for *Campylobacter* in carcasses. This accounted for the number of reported cases in EU in 2011, the ingested dose, the number of servings and the probability of illness per ingested cell. The establishment of a PO was applied to carcasses collected immediately after chilling. Four different datasets were built based on a selection of representative risk factors: slaughterhouse capacity, thinning method, and time between sampling and analysis. The last dataset illustrated the impact of high and low *Campylobacter* contamination for various EU countries on the estimation of POs. Uncertainty in prevalence was assumed to follow a Beta distribution (Uyttendaele et al., 2006) and the Maximum Likelihood Estimation method for censored data was applied to estimate log normal distributions for *Campylobacter* concentration. Subsequently, a scenario analysis with various log reductions (R) was applied to represent for different household thermal treatments. Different fixed values for initial concentration were plotted against the 95^{th} percentile of the final concentration of the pathogens in the food at the time of consumption. The POs were determined by interpolation in the resulting curve. Each model simulation was performed with 10,000 iterations.

RESULTS

The estimation of the FSO for *Campylobacter* was set at -1.2 $\log_{10}$ CFU/g. Regarding slaughterhouse capacity, if more than 10^8 broilers are slaughtered per year, PO can be estimated at 2.38 $\log_{10}$ CFU/g assuming 1-2 R before consumption. If 3-4 R would be applied, PO obtained was equal to 3.18 $\log_{10}$ CFU/g. Besides, batches originating from previously thinned flocks can be more at risk of being colonised with *Campylobacter* since when this risk factor was considered, estimated mean concentrations were 1.05 and 2.38 $\log_{10}$ CFU/g for non previously thinned and thinned flocks,respectively. POs derived assuming 3-4 R were 3.76 and 3.06 $\log_{10}$ CFU/g for the datasets tested to meet the FSO. In contrast, no significant differences were found for the time between sampling and analysis (times lower and higher than 36h) since similar PO values were estimated assuming 2-3R (1.95 and 1.99 $\log_{10}$ CFU/g). Finally, the impact of high *Campylobacter* contamination in EU countries (> 2.5 $\log_{10}$ CFU/g) was shown since a reduction in PO

values higher than 1.5 $\log_{10}$ CFU/g is needed to meet the FSO.

CONCLUSIONS AND IMPACT OF THE STUDY

This study provides a validated methodology for the estimation of risk-based metrics based on a quantitative approach and the performance of scenario analyses. Further, derivation of POs at specific points in the food chain can allow food safety authorities to develop specific microbiological criteria according to their efficiency, ease of implementation and cost-effectiveness. This study has been funded under the EU FP7 project BASELINE (www.baselineeurope.eu).

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Risk assessment of mold spoilage: a case of Aspergillus niger and Penicillium expansum on yogurt

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OBJECTIVE

Despite the employment of Good Manufacturing Practices and hygiene control measures in the dairy industry, the problem of development of visible fungal mycelia on yogurt products before the end of their shelf life's has not been completely eliminated. Given the economic implications of spoiled yogurts, the control of spoilage molds on these products continues to be a great challenge for the food quality managers. The spoilage ability of a contaminating spore reflects the facility of the spore to grow on the surface of a yogurt before the end of its shelf life (Dagnas and Membre, 2013). However, the growth of a fungal spore on a yogurt is a dynamic phenomenon which depends on the time-temperature history of each product (Gougouli and Koutsoumanis, 2010, 2012, Gougouli et al., 2011). From a Quantitative Microbial Risk Assessment perspective the risk of mold spoilage relies upon several factors, such as the species, the biological variability of spores, the time-temperature profile of product's exposure, and the decision about the shelf life or failure as well. Thus, the objective of this study was to estimate the growth of two spoilage molds on yogurt and as a result the probability of having mold spoilage at the various stages of chill chain.

METHODS

The growth of two spoilage fungi, *Aspergillus niger* and *Penicillium expansum,* on yogurt was calculated using a two phase linear model: $D_t = 0$ for t < lag; and $D_t = ju\text{-}(t\text{-}t_{las})$ for t > lag. For these equations D_t is the mycelium diameter (mm) at time *t; JU* is the growth rate (mm/h), *t* is the elapsed time (h); and *t^* is the is the time when the lag phase ends (h). The growth rate for the two molds at certain temperature was calculated based on the Cardinal Models with Inflection (CMsI) for the effect of temperature developed by Gougouli et al. (2011). The lag time at certain temperature was calculated from the equation $1/lag = y(T)/lag_{ref}\text{-}y(T_{ref})$. The gamma factor y(T) was derived from the CMsI for the effect of temperature on lag time developed by Gougouli et al. (2011), and the selected reference temperatures (T_{ref}) were 35°C and 25 °C for *A. niger* and *P. expansum*, respectively. The lag time variability was incorporated in the latter model by using as input the distributions of lag time at reference temperatures based on the findings of Gougouli and Koutsoumanis (2013). The time-temperature data used for the stages of chill chain transportation to retail, retail storage and domestic storage as an input to the growth models were generated in the study of Koutsoumanis et al. (2010). The growth of each fungus was calculated using the Monte Carlo simulation technique with (10,000 iterations) using @Risk 6.1.

RESULTS

Attempting to predict the growth of *A. niger* and *P. expanusm* on yogurt from production to consumption a probabilistic modelling approach was used. Distributions of time-temperature conditions during the different stages of the Greek chill chain were used as inputs to the growth models, while the important source of lag time variability of fungal spores was also incorporated into the models. The total lag time for each fungus, a significant parameter for the output of the model, was determined through a cumulative approach based on the findings of Gougouli and Koutsoumanis (2010). The structure of the developed probabilistic models allows the evaluation of fungal growth and, furthermore, the probability of having mold spoilage during the each stage of the current chill chain separately. Based to these stochastic models, different intervention scenarios for the shelf life were tested in order to quantify the level of risk of spoilage. Moreover, aiming at

improving the challenge tests applied by the dairy industry for the presence of molds, different sampling plans were examined which can give more realistic results for mold presence.

CONCLUSIONS AND IMPACT OF THE STUDY

The findings of the present study in combination with risk assessment can support the decision making and offers the possibility to improve quality management of yogurt.

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Fit for purpose risk assessment of viral pathogens for a vegetables production enterprise in Greece

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OBJECTIVE(S)

Leafy greens that are consumed raw are increasingly being recognized as important vehicles for transmission of human pathogens including viruses, and have been identified as the source of many viral disease outbreaks. Human enteric viruses can be introduced into the food supply chain during different stages of food production, and there is no strict evidence about the most vulnerable stage for virus contamination. The aim of the present study was a fit for purpose virological risk assessment by integrated virological monitoring of a salad vegetables enterprise in Greece, from production to the point of sale. The study was part of the European FP7 project VITAL(Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains) which aimed to gather data on virus contamination, to provide a basis for subsequent quantitative viral risk assessment and recommendation of control measures.

METHODS

Sampling plans were developed by food safety management and risk assessment experts using background information questionnaires, based on HACCP audit principles and food safety fact finding visits. The sampling was longitudinal and samples from all phases (production,processing and point-of-sale)were collected. A total of 221 samples were collected from a vertical production company located in Western Peloponnesus, which produces lettuces for the Greek market and exports to numerous EU countries. Samples comprised of irrigation water, cattle manure, harvester's hands, toilets, and toilet door handles(production phase), rinsing water(processing phase) and lettuce heads(point of sale phase). Lettuces were sampled from a supermarket supplied by the lettuce producer of the study. Samples were analyzed for Norovirus(NoV, GI and GII) and Hepatitis A virus(HAV) with optimized, standardized detection procedures. In addition to these human pathogenic viruses, the presence of human adenoviruses(hAdV) was examined as indicator for fecal contamination. These monitoring data are currently used in a quantitative microbiological risk assessment model to estimate the associated human health risks and the contribution of potential contamination points to the overall virus contamination. Adenoviruses have been proven as a good indicator tool for a fit-for-purpose viral risk assessment.

RESULTS

Pathogenic viruses(NoV and HAV) were not detected in the analyzed lettuce samples. However, hAdVs were detected at 26.2% in samples of all three phases at 25%, 16.6%, and 30.7%, respectively. The elevated prevalence of hAdVs supports the existence of routes of viral contamination, which pathogenic viruses could

follow. A guidance sheet for preventing contamination of leafy green vegetables by viruses was produced and is available in six languages at the following webpage(http://www.eurovital.org/). Following this guidance will help to reduce contamination of leafy green vegetables with viruses on the farm.

CONCLUSIONS AND IMPACT OF THE STUDY

Once fresh produce is contaminated by viruses, there are no realistic post-harvest risk control measures except cooking which is not an option with ready-to-eat fresh produce (FAO/WHO 2008). It is essential for thorough food safety management that systems are developed whereby viruses can be monitored at critical points throughout food supply chain (D'Agostino et al. 2011). Current HACCP procedures should be reviewed in view of possible viral contamination.

The numbers of samples tested for viral contamination in this study were relatively small, especially considering the expected low prevalence. Furthermore, the monitoring was more likely to detect structural contamination events rather than episodic contamination events. Therefore, sampling points that tested negative might be important for episodic viral contamination nevertheless. Conclusively, the study verified the existence of leafy vegetable virus contamination routes, demonstrated the usefulness of index viruses as a tool for tracing the source of fecal viral contamination, and finally underlined the need for future similar fit-for purpose studies for the integrated management of food-borne viral diseases in Europe and worldwide.

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Quantitative exposure assessment to Listeria monocytogenes in uncooked pressed cheeses with a long ripening based on challenge-test data.

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OBJECTIVE(S)

Based on challenge-test experimental data reproducing the behaviour of *Listeria monocytogenes* during the production process of an uncooked pressed cheese with a long ripening (unpublished), the aim of this study was to assess the exposure to this pathogen during the shelf-life of the cheeses, for different contamination scenarios of the raw milk.

In this purpose, a Monte-carlo simulation model was built using data provided by dairy plants producing this cheese and challenge-test data.

METHOD(S)

Data used for the building of the exposure assessment model were:

- experimental challenge-test data reproducing the evolution of *L. monocytogenes* in the cheese, made with experimentaly contaminated raw milk, during cheese making, throughout ripening and storage under different packaging process ;
- technological and physicochemical characteristics of cheeses provided by several dairy plants, integrating the variability of the production process.

The cheese processing was divided in several steps defined according to the production process, the evolution of the physical and chemical parameters, and the results of the challenge-tests. Primary and secondary microbiological predictive models were used to simulate bacterial growth during cheese making and until 30 days of ripening. Optimal growth rates used were 1.2 h^{-1} during the curd step and the first pressing, 0.35 h^{-1} during maturation and acidification steps and 0.18 h^{-1} in the core of cheeses until 30 days of ripening. Then, stabilization and decreasing of *L. monocytogenes* during latter ripening and during storage under packaging were simulated using decreasing rates assessed from challenge-tests data.

Different contamination scenarios of the raw milk were applied to the simulation model: contaminated raw milk with 2 different concentrations and 2 types of milk. Different process options were tested, such as a milk maturation step or not, 8 different steps of packaging and 2 different shelf-lives.

RESULTS

The levels of *L. monocytogenes* predicted by the model from technological and physicochemical data of challenge-tests were in agreement with those obtained in the core of cheeses in the challenge-tests, for the same raw milk contamination levels. However, the model slightly overestimated the levels of *L. monocytogenes* in the rind.

Concerning contamination scenarios simulated, for example with an initial level of contamination of 1 cfu/100 mL of milk, the model predicted average concentrations comprised between 0.795 and 1.273 $\log_{10}$

cfu.g^{-1} in the cheeses after 30 days of ripening (with 90% of core and 10% of rind), according to process options. Then, the average concentrations decreased to values comprised between 0.105 and 0.840 $\log_{10}$ cfu.g^{-1} in 90 days old cheeses. When these cheeses were packaged at 90 days of ripening, the concentrations reached values comprised between -0.730 and 0.442 $\log_{10}$ cfu.g^{-1} at the end of the storage under packaging, according to process options and shelf-lives (21 or 45 days).

CONCLUSIONS AND IMPACT OF THE STUDY

The modelling approach is useful to simulate the evolution of *L. monocytogenes* in the case of low contamination levels of the raw milk, frequently encountered with natural contaminations (and not easily reproducible in challenge-tests) and to integrate the variability of the cheese production process regarding physical and chemical characteristics of the cheeses.

The study pointed out the interest to couple the challenge-test approach and the modelling approach but also the need to develop predictive models for destruction under athermic conditions.

All these data will be useful for food administrative authorities to manage sanitary alerts and to evaluate the appropriate category of these cheeses regarding the European regulation 2073/2005.

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The "Quantitative Microbiological Risk Assessment" Approach In The French Dairy Sector

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OBJECTIVE(S)

In case of contamination, some dairy products may allow the development of pathogenic bacteria. The manufacturer has to guaranty the safety quality of its products for consumers (EC Regulation No. 178/2002). The application of Good Hygiene Practices, the HACCP approach and traceability are implemented tools to prevent microbiological hazard contamination.

A large number of microbiological analyses are performed regularly on milk and on dairy products, from the farm level to the end of the production process. Statistical analysis of these data can provide useful information for the management of the raw milk quality and provide inputs for Quantitative Microbiological Risk Assessment (QMRA) model to assess the behavior of pathogenic bacteria during the process, assess the associated risk of illness for consumers and optimize management options.

The French dairy sector has developed tools to help dairy manufacturers in this purpose.

METHODS(S)

The first tool is a statistical toolkit using microbiological data analysis of raw milk. Data inputs are non-conformity results regarding a given Microbiological Criterion (MC) (e.g. presence or not of *Salmonella* or concentration greater than 300 CFU/ml for *Staphylococcus aureus* in a raw milk sample at the farm level).

Adapted statistical techniques, such as exact confidence interval, Cochran-Armitage test, generalized score test, are used to analyze the data according to a pre-defined procedure. Taking into account the structure of the milk collect constituted by several farms, following specific results can be obtained:

- Annual and monthly prevalences of non-conformity are computed and temporal trends are evaluated. The repartition of farms in safety quality clusters (e.g. cluster 1: no non-conformity per year, cluster 2: at least one per year) can also be provided.
- Correlation between two MC can be identified and the relationships can be quantified (e.g. prevalence of 25% for a given non-conformity level of *Staphylococcus aureus* for a herd with no non-conform analysis of *Listeria monocytogenes*).
- The impact of different rules of farm selection on the quality of raw milk is assessed. Different sorting rules are simulated and results are compared based on their cost/effectiveness ratio.

The second tool is the construction of stochastic QMRA models that can be adapted to several cheese technologies and pathogenic bacteria. Inputs can be the outputs of the statistical toolkit for microbiological dairy data analysis described above, the steps of the manufacturing process, physical and chemical parameters during process and challenge-tests results. Monte-Carlo simulations are used to take into account variability and uncertainty. The outputs of the QMRA model are the prevalence of contamination and the concentration of the product at each step of the process, and the risk of illness.

RESULTS

Results obtained with the first tools will help assessing temporal trends and identifying seasonality for the raw milk contamination prevalence, quantifying the efficiency of intervention strategies, optimizing milk sorting, etc. With QMRA results, the food safety manager will be able to optimize safety management options during cheese production process, identify parameters and steps having the most impact on the food safety objectives and the risk level.

CONCLUSIONS AND IMPACT OF THE STUDY

This approach set up by the French dairy sector for professionals should be used as a management tool by the quality managers to make decisions, based on their microbiological routine data, and their knowledge of the process.

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Quantitative risk assessment of Hemolytic and Uremic Syndrome linked to O157:H7 and non-O157:H7 Shiga-toxin producing Escherichia coli strains in raw milk soft cheeses

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OBJECTIVE(S)

Shiga-toxin producing E. coli (STEC) strains may cause human infections ranging from simple diarrhea to Haemolytic Uremic Syndrome (HUS). The five Main Pathogenic Serotypes of STEC (MPS-STEC) identified until now in Europe are O157:H7, O26:H11, O103:H2, O111:H8 and O145:H28. Because STEC strains can survive or grow during cheese-making particularly in soft cheeses [(1-5)], a stochastic quantitative microbial risk assessment model was developed to assess the risk of HUS associated with the 5 MPS-STEC in raw milk soft cheeses and to evaluate the effect of pre-and post-harvest interventions on this risk.

METHODS

The model considers typical raw milk soft cheese dairy plant collecting milk from 31 dairy herds. The model combines three modules: farm module, cheese production module and consumer module. The outcome of the first module is the probability distribution of the level of milk contamination which was assessed using data on within herd animal shedding prevalence [(6)], number of colony-forming-unit (CFU) shed by infected animals [(7)], amount of milk produced by herd, and indirect data about the amount of fecal contamination in bulk tank milk per milking. The level of contamination of milk tank is the input of the second module, which considers a production of 23,000 raw milk soft cheeses (equivalent of 50,000 litres of raw milk). For each iteration, the module estimates the probability that a portion of 25g of cheese is contaminated with STEC or one of the five MPS-STEC strains and, given that a portion is contaminated, the distribution of the number of organisms at the end of production. The optimal growth rates of STEC strains were assessed by fitting primary and secondary [(8)] growth models on data issued from a specific challenge-test experiment. Cardinal values of STEC strains were assessed from a meta-analysis of data extracted from Combase database. The third module assesses the probability and the level of contamination at time of consumption and the associated risk of HUS. The model was run for different scenarios: Baseline scenario without safety management measures and a set of risk management scenarios combining pre- and post-harvest interventions [(9, 10).] The outputs of risk management scenario are compared to the result of the baseline in term of risk reduction. All the modules were implemented using SAS- software (SAS version 9.3 TS).

RESULTS

Impact of the pre-harvest intervention ranges from 76% to 98% of risk reduction with highest values predicted with scenarios combining a decrease of the number of cow shedding STEC and of the STEC concentration in feces. The impact of post-harvest interventions on the risk based level was also tested by applying five Microbiological Criteria (MCs) at the end of the ripening. The five MCs differ on the sample size, the number of samples that may yield a value larger than the microbiological limit and the analysis methods. The risk reduction predicted varies from 25% to 96% by applying MCs without pre-harvest

interventions and from 1% to 96% with combination of pre- and post-harvest interventions.

CONCLUSIONS AND IMPACT OF THE STUDY

The model described in this paper was built using current knowledge and original approaches to characterize prevalence, concentration and behavior of pathogenic STEC at pre-harvest, soft cheese processing and ripening. This model is a tool for food safety risk managers to choose a MC on the basis of the predicted health benefit (HUS Risk Reduction) and the predicted proportion of cheese batches that will not comply with the MC. Our analysis showed that microbiological analysis for STEC is likely to deliver meaningful HUS reductions. Thus, end-product testing for STEC would in some circumstances significantly reduce the public health risk.

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Risk-Benefit based probabilistic assessment software for heat processed foods

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OBJECTIVE

The preservation of food quality during food processing is a key condition for food industries to remain competitive and respond to consumer demand. For that purpose, food industry must mediate among two objectives: preserve the nutritional benefits and ensure a high level of microbiological risk control.

The aim of this study is to develop a user friendly probabilistic tool able to assess quantitatively the risk and benefit of heat processed foods. The tool allows to appraise the risk (food spoilage) associated to survival *Geobacillus Stearothermophilus* and the benefit related to Vitamin C. In the end it proposes the best compromise to preserve vitamins and to control the microbiological risk of food.

METHODS

The industrial risk considered in this study was that of the thermophilic bacterium *Geobacillus stearothermophilus*, recognized as a major source of spoilage in canned foods and frequently detected in cans presenting defects after 7-day incubation at 55°C (André et al., 2012). *G. stearothermophilus* heat resistance parameters (D_{ref}, Z_T and Z_{pH}), used in this study, were estimated using hierarchical Bayesian modeling (Rigaux et al., 2013). Furthermore, nutritional benefit was that of vitamine C. The two bioactive forms of vitamin C were taken into account: DeHydroascorbic Acid (DHA) and Ascorbic Acid (AA). For both cases, activation Energy (Ea) and reaction rate (K) were considered. Monte Carlo based simulations were used in order to set up the destruction of *G. Stearothermophilus* and both AA and DHA of vitamin C (Rigaux, 2013).

RESULTS

The decision making tool developed in this study allows to quantify the risk due to *G. stearothermophilus* and displays the distribution based Monte-Carlo simulation of the decimal reduction number of the considered bacteria. The distribution is given with a target level user-defined. The probability to reach this known threshold is also calculated. At the same time, the tool displays the distribution based Monte-Carlo simulation of the vitamin C percentage reduction ([AA] and [DHA] concentrations). This distribution is given also with a user-specified allegation threshold for vitamin C. The probability to reach the allegation threshold is also given. Color codes are used to help users to decide on the risk-benefit compromise associated to a given heat processed food.

CONCLUSION AND IMPACT OF THE STUDY

A user friendly risk-benefit based probabilistic assessment tool for heat processed foods was developed in this study. The software allows to accurately evaluate and find the best compromise between nutritional quality and microbiological safety for heat processed food products. Monte-Carlo simulation method was performed in order to estimate the probability to reach both user-defined target levels of bacteria and

allegation threshold of vitamin C. This statistical tool could have a significant industrial impact to better assess temperature profiles applied during heat processing, because not only a microbiological risk is put forth but also the nutritional benefit. This tool will be integrated to the heat inactivation module of Sym'Previus.

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Bacillus cereus in dairy product, a simple decision aid tool for risk assessment

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OBJECTIVES

Mascarpone cheese is an unripened, soft spread cheese obtained from thermal-acidic coagulation of milk cream. During the manufacturing process, mascarpone is subjected to heat treatments designed to extend its shelf-life. However, such treatments, being inadequate to inactivate bacterial spores, must rely on correct refrigeration to prevent microbial spoilage or toxicity during storage. When process deviations occur, the FBOs (Food Business Operators) are required to assess the safety of the product. In such cases, accurate prediction of the growth of a specific pathogen of concern would be necessary. The aim of the study was to develop a simple risk assessment tool, based on mathematical model generated on food data, able to predict the *Bacillus cereus* growth during the cooling of soft spread cheese.

METHODS

Spores of three strains of *B. cereus* (wild and registered) were inoculated separately in mascarpone cheese. Contaminated food was incubated at 12, 20, 25, 30 and 34°C (isothermal temperature) to cover the entire biokinetic growth temperatures of pathogen. Two independent replications for each temperature were performed. The model was carried out in two steps. First, for each growth curve, the specific growth rate (μ_{max}) was calculated using DMfit Excel add in, based on Baranyi and Roberts model (1994). Then, the secondary model was obtained by regressing the natural logarithm of observed μ_{max} to the respective temperature. To obtain predictions for the bacterial concentration during time-dependent temperature profiles, the dynamic model was solved numerically using the second order Runge-Kutta method in an Excel® spread-sheet. External validation was carried out using independent data sets considering the cooling profiles of a local dairy company. When comparing the prediction with observation, the initial concentration was taken as the observed inoculum. Bias and discrepancy between model predictions and observations were estimated as reported by Baranyi et al. (1999).

RESULTS

In mascarpone cheese, the μ_{max} of *B. cereus* varied from 0.066 h^{-1} at 12°C to 1.69 at 34°C with a μ_{max} standard error (SE) between 0.001 and 0.339. The adjusted R^2 value for the secondary model was 0.94 with a SE of 0.25. The discrepancy between the predicted and observed data was acceptable, even if the model over predicted the growth of the *B. cereus.* The safe prediction is due to the fact that in the model was considered just the μ_{max} parameter and not the adaptation time to the substrate Accurate quantification is important for estimating the risk for the consumer of a food product, since it is generally believed that food exceeding 10^3 cfu g^{-1} of *B. cereus* is not safe for consumption (Granum, 2002). The model was used to develop an Excel® add in decision aid, useful for FBOs to evaluate the safety of their cooling process, also in case of temperature abuse or process deviations within their HACCP systems. The software will be published on www.ars-alimentaria.it, an Italian food safety database, funded by Italian Health Ministry.

CONCLUSIONS AND IMPACT OF THE STUDY

Predictive models can provide the estimates of the growth of foodborne pathogen in a food matrix in a real

time situation. Such predictive models can also be useful in day to day decision making in the food processing operations, in HACCP implementation and risk assessment in the event of temperature abuse and process deviation. More validation studies are necessary in order to evaluate the efficiency of the obtained predictive models.

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Industrial concerns and needs: Development and application of a predictive model of mold spoilage as a tool to improve shelf life of bakery products

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OBJECTIVES

Predictive modelling and microbial risk assessment have emerged as a comprehensive and systematic approach for addressing the risk of microbial spoilers in specific foods and processes. Within this framework, the level of undesirable microorganisms in the food chain is normally assessed using classical tools and approaches for microbial enumeration and prediction of behavior. In the case of molds, a product is spoiled as soon as mycelium becomes visible, which usually occurs very shortly after lag time. This study aims at the determination of growth cardinal values i) to determine growth/no growth boundaries ii) to predict the appearance time as a function of environmental factors, iii) to validate simulations with experimental challenge tests performed on cakes.

METHODS

One strain of the fungal specie commonly considered as contaminant of pastries: *Aspergillus candidus* was studied. Rosso model was used to describe and evaluate cardinal values, i.e. minimal, optimal and maximal values of aw and temperature allowing growth on agar media.. Cakes were artificially inoculated to determine minimum time for mycelium to become visible (t_v). In order to take into account the matrix parameter, $1/t_{v\ opt}$ was determined instead of optimal growth rate (μopt). To further validate simulations, an *in situ* study was carried out, involving 51 industrial Madeleine cakes.

RESULTS

Cardinal values of these 4 strains were determined for both, growth boundaries and simulations taking into account aw, temperature and potassium sorbate concentrations. Madeleine cakes challenge tests were used to determine i) observed tv in relevant industrial condition (T°:25°C, aw: 0.8) ii) estimated $t_{v\ opt}$ (matrix parameter) and iii) growth simulations. For a range of water activity (0.7 to 0.9) and temperature (15 to 25° C), a total of 51 cakes were inoculated with *Aspergillus candidus*. For aw of equilibrium (after 2 weeks storage), correlation between simulations and experimental data were satisfactory (R^2=0.85, BF=0.98, AF=1.10). These results were validated for pastries but could be enlarged to other food to simulate the impact of product formulations and storage conditions.

CONCLUSIONS AND IMPACT OF THE STUDY

This study shows that predictive models developed for bacterial growth simulation are successfully applied to describe the apparition of moulds on bakery products. For characterized strains, only one challenge test condition is needed to estimate appearance time in any conditions of storage. This approach is further useful for food microbiologists and manufacturers whose aim is to predict the likelihood of fungal spoilage as well as the development of new formulations minimizing fungal growth. The transfer of bacterial predictive concepts to fungal contaminant is currently in progress for both growth and destruction of super spoilers or diehard microorganisms.

Survey of Listeria monocytogenes in Ready-to-Eat Foods in the United States (Phase I): Assessing Potential Changes in a Decade

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OBJECTIVE

Although significant efforts have been taken over the last decade to control *L. monocytogenes* in ready-to-eat (RTE) foods, a well-designed survey is needed to determine whether changes occurred in its prevalence and levels and to provide current data to assess the relative risk of listeriosis associated with the consumption of higher risk foods. A multi-agency, multi-disciplinary study was undertaken to determine the current prevalence and levels of *L. monocytogenes* in RTE foods purchased at retail establishments in four FoodNet sites in the U.S.

METHODS

The study was designed in two phases. Here, we report the results from phase I, which included seven FDA regulated product categories: smoked seafood, seafood salads, low acid cut fruits, soft ripened and semi-soft cheeses, deli salads, raw milk, and prepared sandwiches. Samples were collected in both supermarket chains and independent grocery stores (*ca.* 400 retail stores) in California, Maryland, Connecticut and Georgia on a weekly basis for 50 weeks between December 2010 and January 2011. Information was recorded for the types of product and store as well as the ingredients. Samples were analyzed using the FDA-BAM methods and included screening (25 gram or ml per each sample in enrichment broth tested by the BAX Assay for *L. monocytogenes*; BAX® System Q7), and enumeration of positive samples by a three-tube four-level MPN method (FDA, 2003 and 2006). *L. monocytogenes* isolates were obtained following isolation and biochemical confirmation using procedures described in Gombas et al. (2003) with modifications.

RESULTS

Of 7,917 samples tested, 46 samples tested positive by the BAX assay for *L. monocytogenes*; *L. monocytogenes* was isolated from 40 samples. Among the seven product categories, between zero (for soft ripened cheese, n=2028) and1.04% (for deli-type salads, n=1347) of samples tested positive for *L. monocytogenes* (with isolates), and between 1.23 and 5.99% tested positive for *Listeria* spp. Further analysis is underway to determine *L. monocytogenes* prevalence, taking into account potential clustering effects. For the *L. monocytogenes* positive samples, levels ranged from <0.03 MPN/g to >110 MPN/g. Compared to manufacture-prepackaged samples, higher fractions of deli-made samples were found to be contaminated. For example, for deli-type salads, in this study we found 1.41% (deli-made) vs. 0.53% (prepackaged) positive by the BAX *L. monocytogenes* assay, compared to 3.6%(deli-made) vs.1.4% (prepackaged) that had been reported in a comparable study in the U.S. a decade ago (Gombas et al. 2003).

CONCLUSIONS AND IMPACT OF THE STUDY

The overall likelihood of contamination seems to have decreased for *L. monocytogenes* in the RTE foods in the survey. However, the fact that *Listeria* appears to be more likely detected in deli-prepared than prepackaged samples remains a challenge. Our findings provide data needed to assess changes in *L.*

monocytogenes prevalence and levels in RTE foods and will be used for risk assessments to inform policy decisions. The study also underscores the importance of continued research to develop and validate interventions to ensure a safe food supply.

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Predictive Microbial Modelling for the tenacity of Escherichia coli in Lettuce using the open tools PMM-Lab and FoodProcess-Lab

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OBJECTIVE(S)

Predictive microbiology is an important part of quantitative microbial risk assessments. Software tools help to estimate primary and secondary models from pathogen specific laboratory data based on given mathematical formulas. The Predictive Microbial Modeling Lab (PMM-Lab) and the FoodProcess-Lab tools have been developed to serve as a community resource and are free to use. The main advantage of these tools is, that raw data used to generate predictive microbial models and the model generation workflow itself remain connected with the final model. In addition, the tools can be downloaded and used offline keeping unpublished data on the desktop. Here, we apply both tools for the estimation of growth parameters for verocytotoxigenic *E. coli* in lettuce in order to predict the growth from the point of harvest to the point of sale in the supermarket. Results are compared with previously published models and show the validity of the two software tools.

METHODS(S)

Raw data for the growth of verocytotoxigenic *E. coli* in lettuce were used from the ComBase (www.combase.cc). Mathematical formulas for the generation of growth models were gathered from the literature. Data and formulas were imported into the PMM-Lab software, http://sourceforge.net/projects/pmmlab/). There, primary and secondary models were fitted to the experimental data. The estimated models were applied in the FoodProcess-Lab software (http://sourceforge.net/projects/foodprocesslab) to calculate the growth of *E. coli* in lettuce in different processing and storage steps covering the time at harvesting up to the supermarket. The temperature curve was measured by Koseki et al. (2005). Generated modelling results were compared with predictions from the Pathogen Modeling Program (PMP, http://pmp.arserrc.gov/PMPOnline.aspx) and from Koseki et al. (2005).

RESULTS

The fitting of the primary model (Baranyi et al., 1995) with PMM-Lab to the growth data of verocytotoxigenic *E. coli* in lettuce published by Koseki et al. (2005) was in good agreement with those from PMP. For µmax, a value of 0.06, 0.17, 0.40 and 0.53 were estimated for 10, 15, 20 and 25°C, respectively (R^2 = 0.99, 0.94, 0.96 and 0.97). The dependency of µmax from temperature was modelled by application of the Ratkowsky secondary model equation (Ratkowsky et al., 1982) (R^2 = 0.97). Here, the factor b was equal to 0.031 and thus slightly lower than that of PMP (b = 0.032) and Koseki et al. (b = 0.033).
The growth of verocytotoxigenic *E. coli* from harvesting to the point of sale was estimated with the combination of the Baranyi and the Ratkowsky models. According to PMM-Lab, cell counts rise from log 3.3 to log 4.1 and thus increase slightly lesser than estimated by Koseki et al. (max = log 4.2) and PMP (max = log 5.0).

CONCLUSIONS AND IMPACT OF THE STUDY

The results generated with the software PMM-Lab and FoodProcess-Lab are in good agreement with studies published before. The main benefit of the application of PMM-Lab and FoodProcess-Lab is the transparency

generated over the whole model generation process. Additionally, as both tools are freely available to any user this approach could pave the way to better exchange predictive microbial models within the scientific community. In addition – as the output of both tools can be displayed in auto-generated reports – the tools PMM-Lab and FoodProcess-Lab can serve food producers as valuable tools for quality control measures by reading out data logger and estimating growth of pathogens in real time.

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Quantitative Microbial Risk Assessment for safe food and food security

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OBJECTIVE

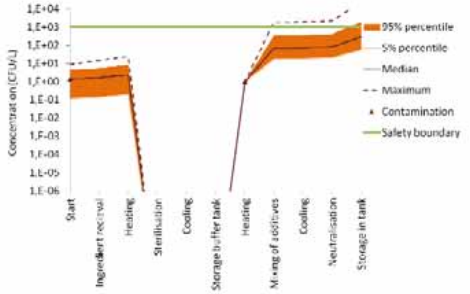
Current trends in food manufacturing give the industry new challenges in food production:

- For reasons of globalization and scarcity, manufactures purchase a wide variety of raw materials and ingredients with also a variety in microbial quality.
- Sustainability targets stimulate companies to use more clean label ingredients and materials from other production chains with their own microbial dynamics.
- The tendency to use less salt and sugar in food products asks for additional (processing) measures to prevent outgrowth of spoilage micro-organisms.

The aim of our research is to provide the industry with a validated tool to predict the probability of food spoilage as a function of raw material quality, process design and operation and accidental contamination.

METHODS

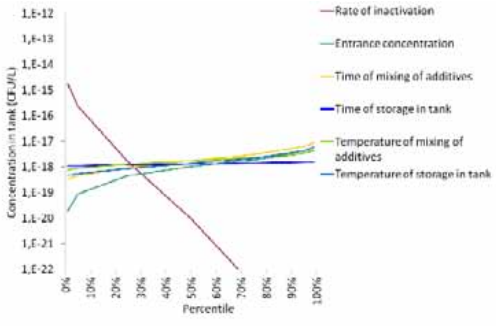
Quantitative Microbial Risk Assessment (QMRA) is a common method to assess a production chain on microbial risks. A combination of microbial kinetics, processing parameter, statistical techniques (Monte Carlo simulation) and mathematical modeling leads to the estimated risk of food contamination and a list of most influential parameters. As a next step in quantitative risk assessment QMRA is used in combination with other tools focusing (i.e. NIZO Premia) on other quality attributes related to flavor and texture, and production costs. To provide the industry with a sound prediction and advice, big datasets of, for example, growth and inactivation kinetics are used. In this way also strain variability can be included. Production chains have been simulated from farm to fork.



RESULTS

Starting with the dairy industry, several production chains have been evaluated. Typical outcomes are:

- a concentration profile of target organisms along the production chain, including 5-95% percentiles.
- a sensitivity analysis of several parameters of the production chain or target organism and its effect on food contamination.



CONCLUSIONS AND IMPACT OF THE STUDY

Using QMRA in industrial food production chains leads to a focus on the most influential parameters in a complex chain of events and to a fact based discussion. The industry benefits from reduced sampling costs, reduced re-calls and convinced authorities.

Risk ranking of ready-to-eat foods of non-animal origin in the EU

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OBJECTIVE

Food of non-animal origin (FoNAO) are a major component of almost all meals and comprise a wide range of fruit, vegetables, salads, seeds, nuts, cereals, herbs, spices, fungi, and algae. Given the importance of outbreaks caused by contaminated foods of non-animal origin (FoNAO), there is a need to evaluate the establishment of specific control measures for certain FoNAO supplementing the general hygiene rules.
Therefore, the European Commission asked the European Food Safety Authority (EFSA) to identify and rank specific food/pathogen combinations most often linked to foodborne human cases originating from food of non-animal origin in the EU (EFSA, 2013).

METHODS

A risk ranking model based upon a Risk Ranking Tool (RRT) published by the U.S. Food and Drug Administration (FDA) (Anderson et al., 2011) was developed. This model was based on a semi-quantitative risk ranking algorithm that orders the priority of pathogen-commodity combinations according to seven criteria: (i) strength of associations between food and pathogen based on the foodborne outbreak data from EU Zoonoses Monitoring (2007-2011), (ii) incidence of illness, (iii) burden of disease, (iv) dose-response relationship, (v) consumption, (vi) prevalence of contamination and (vii) pathogen growth potential during shelf life.

For each criterion, the available data were grouped into scoring categories, which were defined and assigned a numerical, ordinal score. The definition of the scoring categories was based on the data available and finally reviewed by expert opinion. For each food/pathogen combination, a reference score was calculated by a summation of the scores from all seven criteria.

RESULTS

The top five ranking groups of food/pathogen combinations were, in decreasing order of priority: (i) *Salmonella* spp. and leafy greens eaten raw as salads; (ii) *Salmonella* spp. and bulb and stem vegetables; *Salmonella* spp. and tomatoes; *Salmonella* spp. and melons; and pathogenic *Escherichia coli* and fresh pods, legumes or grain; (iii) Norovirus and leafy greens eaten raw as salads; *Salmonella* spp. and sprouted seeds; and *Shigella* spp. and fresh pods, legumes or grain; (iv) *Bacillus* spp. and spices and dry powdered herbs; Norovirus and bulb and stem vegetables; Norovirus and raspberries; *Salmonella* spp. and raspberries; *Salmonella* spp. and spices and dry powdered herbs, *Salmonella* spp. and leafy greens mixed with other fresh

FoNAO; *Shigella* spp. and fresh herbs, pathogenic *Escherichia coli* and sprouted seeds; and *Yersinia* spp. and carrots and (v) Norovirus and tomatoes; Norovirus and carrots; *Salmonella* spp. and nuts and nut products and *Shigella* spp. and carrots.

CONCLUSIONS AND IMPACT OF THE STUDY

The model outputs are based on the reported outbreaks associated with consumption of FoNAO within the EU between 2007 and 2011. Thus, the model is likely to underestimate the importance of diseases which appear to be of a more sporadic nature (such as those due to *Listeria monocytogenes*, *Campylobacter* spp. and parasites). In addition, future fluctuations in the reported outbreaks are likely to impact on the ranking orders. Using the risk ranking model on a regular basis with updated data will provide a tool that may also show trends in the importance of different food/pathogen combinations.

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Assessment of the impact of consumer behaviors on exposure to Listeria monocytogenes by deterministic and stochastic approaches

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OBJECTIVE(S)

Previous risk assessments of listeriosis in ready-to-eat (RTE) foods showed that variability of storage temperatures and duration in the domestic refrigerator are important (Ellouze et al., 2010). The variability of these parameters which are related to consumer behaviours is generally based on survey data or product temperature measurement. However, these data are only descriptive and it is difficult to link them to practical information such as ambient temperature or thermostat setting point.

This study aims to propose a new approach combining the deterministic and stochastic modelling to predict the product time-temperature evolution along the logistic supply chain and its impact on the growth of *L. monocytogenes*. The variability of time-temperature histories was modelled according to several parameters such as positions in the display cabinet, external air temperature, type of refrigerators. Then, the developed model was used to assess the impact of consumer behaviours on listeriosis exposure.

METHODS

The time-temperature histories of the product along the cold chain was predicted using the methodology developed by Flick et al.(2012). This study proposes a general methodology combining determinist models for equipment and product evolution and stochastic models to consider different sources of variability of the cold chain (sequence of equipments, ambient conditions and thermostat setting temperature). The studied product, called 'product of interest', can follow different itineraries (type of equipment, position and residence time). This methodology was applied to the pre-package meat cold chain (Hoang et al., 2012) and the survey data in France (ANIA, 2004) were used to compare with the result of simulation.

In this study, the growth of *L. monocytogenes* in cooked ham was predicted from the display cabinet to the consumption. The output parameter of the model is the bacterial contamination at the consumption point y_{end} (log_{10}cfu/g). The probability of non- to a FSO (Food safety Objective) was calculated for a given residence time and product temperature in equipments using an accept-and-reject algorithm (Guillier et al 2011). The FSO was fixed to 100 CFU/g at the consumption point relatively to European Union regulation.

Parameters related to consumers behaviour (refrigerator thermostat setting, use of insulated/non insulated shopping bag, product position in equipments, product consumption before or after shelf life …) and environmental conditions (ambient temperature due to season, geographical location…) were studied with respect to the probability of non-compliance to the FSO.

RESULTS

The parameters related to consumer behaviors such as the residence time and the thermostat setting have a

great impact on the final product contamination. A potential increase of one notch of the thermostat setting point in the domestic refrigerator would improve significantly consumer's safety. Moreover, no difference was observed if the product is picked at the rear and the front position in the display cabinet. The impacts of temperature and residence time in a shopping bag, the ambient temperature variation due to season and geographical location appear to be limited.

CONCLUSIONS AND IMPACT OF THE STUDY

Results showed the importance of consumer behavior on food safety. The numerical tool developed in this study could be used by food business operators to assess the impact of the modification of the cold chain logistic or by public organization to give the most pertinent and concrete instructions to consumers.

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ADVANCES IN METHODS AND MODELS IN PREDICTIVE MICROBIOLOGY

Oral conferences

Global control of gene expression in Escherichia coli

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The adaptation of the physiology of the enterobacterium *E. coli* to environmental fluctuations involves system-wide changes of gene expression. This reprogramming of the cell takes place at two different levels: on a global scale through the adjustment of the level and activity of the components of the gene expression machinery (RNA polymerase and ribosome); and locally through the adjustment of the concentrations of regulators specifically coordinating the cell response to the new environmental conditions.

Classical studies in bacterial physiology have shown the close interactions between these two levels of regulation in the adaptive response of bacteria. Most work in systems biology has focused on gene networks with transcription factors and other specific regulators, whereas the modeling of the gene expression machinery and its interactions with other regulatory mechanisms has received less attention until now. In this presentation, I will give an overview of current work on the modeling of the gene expression machinery and present recent experimental and modeling results from our group.

Delphine Ropers received a MSc degree in microbiology and enzymology from the Henri Poincaré University in Nancy (France), where she completed a PhD thesis in structural, cellular, and molecular biology in 2003. She studied, both by experiments and mathematical modeling, the splicing regulation of the human immunodeficiency virus RNA. Then, she joined INRIA, where she is currently a research scientist in the Ibis group at INRIA Grenoble—Rhône-Alpes. She applies a systems biology approach to understand survival strategies of the model bacterium Escherichia coli in situation of nutritional stress. This includes analyzing the dynamic functioning of biochemical networks within the bacterium by means of a combination of modeling and experimental tools. She is also a member of the board of the Complex Systems Institute in Lyon (France).

Integrating different –omics levels to model a metabolic switch of E. coli during osmotic stress

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OBJECTIVES

One of the challenges of systems biology for predictive modeling is to find a balance between robust parsimony and integrating relevant elements from disparate sources, possibly even different scales (Pin et al., in press). According to the literature, the growth of *E. coli* under osmotic stress may be limited by physical factors such as the crowding of the cytoplasm (Cayley and Record Jr., 2003), decreased metabolic efficiency as a consequence of the cost of the cell regulation (Csonka and Epstein, 1996) etc. We have previously shown that the known metabolic adjustments alone cannot account for the decrease in growth rate as more and more NaCl is added (Metris et al., 2012). We have also shown that, in the presence of an osmoprotectant, in minimal medium with NaCl between 4 and 5%, there is a metabolic switch (Metris et al., submitted). The aim of this study is to characterise the metabolic state of *E. coli* as a function of NaCl concentration, based on –omics measurements at the genomic scale. We integrate the observed metabolic and regulatory features and propose a model, which can explain the switch.

METHODS

Escherichia coli K12, strain MG1655, was grown in Basic Minimal Medium (BMM, Zhou et al., 2011) with glycine betaine at NaCl concentrations ranging from 2% to 5.5%. The growth rate and "metabolic efficiency" concerning various nutrient sources was determined by optical density in a Bioscreen, at 37^{o}C, as in Metris et al. (submitted). When the maximum cell density was below the detection level of the Bioscreen, the kinetics parameters were determined by plate counts. Data on relative enzyme concentrations were obtained by 2D gel electrophoresis spot volume comparative analysis and Mass Spectrometry based protein identification. The metabolite concentrations in the cell and in the supernatant were determined by proton Nuclear Magnetic Resonance (Rabinowitz and Kimball, 2007). The metabolic state of the bacteria was modelled at the genomic level, using the stoichiometric model of Feist et al. (2007). Inferences on regulation were obtained from RegulonDB (http://regulondb.ccg.unam.mx/).

RESULTS

At the genomic scale, we found that the regime switched from fast aerobic growth to anaerobic/micro-aerophilic fermentative metabolism between 4.5 and 5% NaCl. To model the bacterial strategy to cope with osmotic stress, we defined two objective functions (functions to optimise) for the different regimes. We assumed that the aerobic metabolism corresponds to a maximisation of the growth rate, the optimisation of the kinetics parameters of intracellular biochemical reactions. The objective function in the latter regime was interpreted as an optimisation of the utilisation of nutrients. This is the domain where Flux Balance Analysis (Feist et al., 2007) is a suitable tool, because the route to produce the biomass most efficiently from available nutrients is optimised based on stoichiometric coefficients of the metabolic reactions.

We propose a reduced model which assumes that the metabolic switch is the result of cellular iron homeostasis. The final model is an optimisation problem, constraints of which integrate biochemical reactions, primarily characterised by their kinetics parameters, and feedback loops (Goel et al., 2012).

CONCLUSIONS AND IMPACT OF THE STUDY

In this study, we integrated data and theory of different nature and level to model the behaviour of *E. coli* as a function of osmotic stress. We demonstrated that systems biology approaches are suitable to develop accurate models of bacterial responses to stress and that predictive microbiology is "not only about the rate" (McMeekin et al., 2013). We expect that as more experimental data are made available, the model can be adjusted to capture other relevant stress response features. Our approach hopefully shows that more fundamental interdisciplinary science can help predictive microbiology.

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Predictive microbiology combined with metagenomic analysis targeted on the 16S ribosomal DNA: A new approach for food quality

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OBJECTIVES

The food spoilage process is mainly caused by alteration micro-organisms and classical culture-based methods have therefore been used to assess the microbiological quality of food. These techniques are simple to implement but may not be relevant to understand the modifications of the microbial ecology which occur in the food product in response to different changes in the environmental conditions. Metagenomic analysis targeted on 16S ribosomal DNA can bring about a solution to this new need and elucidate microbial community structures, including the identification and quantification of culturable and non-culturable organisms, at a much higher resolution than was previously possible with culture-based methods to provide a picture of the microbial community. Combined with predictive microbiological models, a new approach was investigated to take into account the dynamics of the evolutions of the microbial community in food products. This work describes the application of a metagenomic analysis and predictive microbiology in order to study bacterial populations dynamics in perishable foods under different environmental conditions.

METHODS

White pudding samples, a typical Belgian pork meat product, were packed under food wrap (atmospheric air condition). Durability studies were conducted at 4°C, 12°C and a dynamic temperature profile according to the NF V01-003 standards (4°C (1/3 of the shelf life) - 8°C (2/3 of the shelf life)) during 15 days. The effect of organic acids was also investigated using a lactic acid / diacetic acid mix (1.8% w/w) treatment. At each day of the trials, classical microbiological (total flora, lactic acid bacteria) and 16S rDNA metagenomic analysis were carried out on all these samples. For the metagenomic analysis, a sequencing library was generated, targeting the V1-V3 region of the 16S rDNA. Libraries were sequenced on a GS junior sequencer using Titanium technology. The Bio-informatic pipeline using Mothur, Blast and Stamp was used to assign a taxonomical identity to the sequences and to obtain the bacterial population proportions of the samples (Schloss, Westcott et al. 2009). The major bacterial populations were thus identified and predictive microbiology models (Baranyi and Roberts 1994; Augustin, Zuliani et al. 2005) were used to assess the growth parameters. The model was validated using the data obtained at a dynamic temperature profile.

RESULTS

The metagenomic analysis of the samples shows that the bacterial populations from the day 0 sample to the post-shelf life sample have important modifications. *Brochothrix* and *Psychrobacter* were identified as the dominant flora. As expected, the storage temperature had a strong impact on the bacterial evolutions. Moreover, the use of lactic acid/diacetic acid reveals the sensitivity of the different populations to the treatment. For the storage at 4°C, the initial dominance of *Pseudomonas* and *Shewanella* is slightly reduced during storage until shelf life, after which it drops to be replaced by *Brochothrix* and *Psychrobacter*. The addition of the preservation treatment has a statistical negative impact on the *Psychrobacter* and

Acinetobacter populations. During the ageing assay (2 days at 4°C followed by 10 days at 8°C), the analysis underlines the influence of the temperature change on the onset of the *Brochothrix* and *Psychrobacter* dominance compared to the entire 4°C storage. Again, the preservation treatment delays this onset. Finally, at an abusive 12°C temperature, samples are quickly dominated by the *Psychrobacter/Brochothrix* pair after 2 days of storage. In this case, the lactic acid mix does not appear to be of any effective use.

Adjustment of primary model was made on the major bacterial populations and simulation was made based on estimated growth rate. The simulations of the three major populations seem to be sufficient for this food product to predict 80 -90 % of the bacterial population at the end of the shelf life in function of the environmental conditions.

CONCLUSIONS AND IMPACT OF THE STUDY

Compared to culture based methods on selective media and previous independent culture techniques, metagenomic analysis combined with predictive microbiology gives more valuable information, and its use could be considered as a technique for quality control or for accurately determining shelf life.

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Understanding the diversity of dairy L. lactis subsp. lactis strains in milk applications: Genome, transcriptome and phenotype integration

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OBJECTIVE(S)

The mesophilic lactic acid bacterium *Lactococcus lactis* is used in particular as starter culture in the manufacture of dairy products. *L. lactis* subsp. *lactis* strains determine preservation (acidification and coagulation preserve milk from unwanted bacteria or mould growth) and organoleptic quality (small quantities of Volatile Organic Compounds [VOCs] and enzymes contribute to aromas and flavors) of the fermented products. Efficient starter selection requires an assessment of the natural diversity of *L. lactis* subsp. *lactis* subpopulation. Understanding the determinants of this bacterial diversity is a prerequisite for predictive approaches. In this work, we investigated the intrasubspecies diversity of *L. lactis* subsp. *lactis* strains exhibiting a dairy phenotype (efficient growth in milk and high acidification rate) with an integrated approach based on a multilevel analysis at genomic, transcriptomic and phenotypic levels.

METHOD(S)

Thirteen strains of *Lactococcus lactis* subsp. *lactis* were studied. Both the genetic (MultiLocus Sequence Typing, MLST) and genomic (Pulsed-Field Gel Electrophoresis, PFGE) levels of the diversity were investigated. These strains were grown in an ultrafiltration cheese model or in a pasteurised standardised cow's milk. For a subset of strains, an array-based Comparative Genome Hybridization (CGH) was achieved to identify common genes and to analyse their expression. Transcriptome was performed under UF-cheese model conditions by using DNA arrays at 24 h of growth. Acidification and growth abilities were monitored throughout the cheese ripening process. To assess phenotypic diversity, an exhaustive phenotypic characterisation was performed in pasteurised milk for nine strains. Eighty-two variables selected as important dairy features were measured; they included physiological descriptors (growth, acidification), and the production of various metabolites and VOCs.

RESULTS

Even if the studied strains displayed large genomic similarities, sharing a large core genome, the expression of this core genome directly in the cheese matrix revealed major strain-specific differences. This work demonstrated that significant transcriptomic polymorphisms exist even among *Lactococcus lactis* subsp. *lactis* strains with the same dairy origin (1). Principal component analysis (PCA) of nine genetically closely related strains demonstrated the phenotypic uniqueness of each of strains based on 82 variables (2). We developed a statistical method of variable selection and 20 variables were selected, all associated with VOCs, as phenotypic markers allowing discrimination between strains. These markers are representative of the three metabolic pathways involved in flavour: lipolysis, proteolysis and glycolysis. Despite the large phenotypic diversity, the strains could be divided in four robust phenotypic clusters depending on their metabolic orientations. Surprisingly, the four strains in our sample belonging to biovar *diacetylactis* were not grouped in a single cluster. New insights into *L. lactis* diacetyl- and acetoin-producing strains isolated from diverse origins indicated that this aroma production is not restricted to strains able to grow in milk and highlighted the genetic and metabolic differences of these diacetyl producing strains (3). Inclusion of genotypic diversity (MLST and PFGE) in addition to phenotypic characters led to a more sensitive diversity analysis (five clusters rather four being defined). However, genotypic characters have a smaller contribution than phenotypic characters (no genetic distances selected among the most contributory variables). This work proposed an original method for phenotypic differentiation of closely related strains in milk and may be the first step towards a predictive classification for the manufacture of starters.

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Selection of mRNA biomarkers using Partial Least Square algorithm to further predict Bacillus weihenstephanensis acid resistance

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OBJECTIVES

Today, food predictive microbiological models have been mainly developed using culture based methods and worst case *scenari* to ensure food safety. It is now commonly recognized that this yields to over-estimation in microbial risk assessment, and could be improved by integrating the bacterial adaptive responses implemented and associated physiological mechanisms. Jointly with the development of bioinformatics, Omic technologies are expected to play an important role in understanding how bacteria can adapt to stressing conditions. In order to integrate Omic data to quantitative microbiological risk assessment in foods, gene expressions may serve as bacterial behaviour biomarkers. In this study an integrative approach encompassing predictive modelling and mRNAs quantifications, was followed to select molecular biomarkers to further predict the acid resistance of *Bacillus weihenstephanensis*.

METHODSS

A multivariate analysis, based on Partial Least Square algorithm (PLS), was performed to correlate the acid bacterial resistance (first decimal decrease time at pH4.6) and the gene expression of vegetative cells with or without exposure to stressing conditions (BHI ± NaCl 2.5%, ± 8°C, ± pH 5.5; ± pH 4.6). This mathematical method provides the advantage to take gene expressions and their interactions into account. A step wise procedure involving PLS, orthogonal PLS (OPLS) and hierarchical PLS (H-PLS) was used to select the most relevant genes among 30 genes for be used as acid resistance biomarkers of *B. weihenstephanensis*. All calculations were performed using Simcap+ version 13.0 software (Umetrics, Umea, Sweden) with unit of variance scaling of the data.

RESULTS

9 genes were selected as biomarkers, and are implied in 3 type of biological answers. 3 genes (*cydA, katA* and *nos*) are implies in general stress response, 3 (*dps, napA* and *rsbW*) in the oxidative stress response and at last 3 (*codY, fabF* and *sigH*) in the metabolic rearrangements. The Variable Importance Projection values obtained by H-PLS for each biological module underlined that each module has equivalent influence in the H-PLS model built, and thus we choose to select the 3 most relevant biomarkers of each biological modules. Furthermore, PLS model could be used as mathematical model to predict the bacterial behaviour. a PLS model based on the absolute quantification of gene expression of the selected biomarkers was built and validated by independent datas. The acid resistance of three samples were predicted to 0.74 h, 0.97 h and 2.08 h whereas the acid resistance observed was, respectively, equal to 0.5 h, 1.3 h and 1.8 h.

CONCLUSIONS AND IMPACT OF THE STUDY

Based on both predictive microbiology and mRNAs quantifications, the response of *B. weihenstephanensis* KBAB4 to acid stress was investigated. PLS based analysis allowed the selection of 9 genes as biomarker of the acid resistance of *B. weihenstephanensis* taking the correlation between biomarkers into account. If the

selection of potential biomarkers offers new perspectives for the prediction of bacterial behaviour, one remaining challenge is to increment these data into mathematical model to further predict bacterial behaviour and thus offer new decision making tools for food safety and quality management. As a firstly described model, the developed concept showed promising results in how integrate these Omics data in bacterial behaviour prediction through the use of mRNA quantification. Moreover an overlap of *B. weihenstephanensis* and *B. cereus* adaptive behaviour was observed supporting the extrapolation of such generic biomarkers and concept to other related species.

Correlating boundaries between survival, growth and expression of genes associated with stress and virulence of Listeria monocytogenes in response to acid and osmotic stress

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OBJECTIVE

Listeria monocytogenes is a rather versatile foodborne pathogen, capable of surviving under stringent conditions and with high adaptative response potential. Hereby, we aimed: (i) to model the impact of various combinations of suboptimal pH and NaCl at low temperature on its the survival/no survival interface of *L. monocytogenes* after exposure to acid stress. (ii) to map the physiological response of *Listeria monocytogenes* at the above conditions at a molecular level, studying the relative expression of stressrelated genes such as glutamate decarboxylase system (gad) sigma factor B (sigB) and positive regulatory factor A (prfA).

METHODS

Two strains of *L. monocytogenes* 6179 (serotype 1/2a) and C_5 (serotype 4b) isolated from a farmhouse cheese and a farm environment respectively, were used throughout this study. Tryptic Soya Broth supplemented with 0,6% w/w yeast extract (TSBYE) served as basal medium. Various combinations of NaCl (0-10 % w/V) and pH (4.8-6.4) were prepared in triplicate. NaCl was added to the basal medium prior to sterilization, whereas pH was adjusted using HCl (6N) post-autoclave. TSBYE without any additional salt or HCl was used as control. The different media were inoculated with 10^7 CFU/ml of *Listeria monocytogenes* and stored at 7^oC for 10 days. Survival under acidic conditions was assessed in TSBYE pH 2.0 (adjusted using HCl) for 5 min. During storage CFU counts, prior and post acid challenge, were determined by plating on TSAYE. Additionally, samples were collected for further molecular analysis. RNA was extracted enzymatically, following phenol-chloroform purification and RQ1 DNase (Promega) treatment. cDNA was synthesized using Superscript II Reverse Transcriptase (Invitrogen) and random hexamers, according to manufacturer's protocol. SYBR green based Real time PCR was performed in order to determine the relative expression of stress and virulence related genes, where 16S rRNA served as reference gene.

RESULTS

Growth/no growth interfaces of strains tested, were determined on different days during storage at 7^oC. Growth for both serotypes 1/2a and 4b was observed at optimal pH values and low NaCl concentrations (0-2% w/V), whereas both strains reached maximum population of 9 log CFU/ml in approximately 10 days. Nevertheless, strain C_5 was more affected by the presence of low pH (≤ 5.5) and high NaCl concentration (8-10%), resulting in 4-6 log reductions on day 10, while strain 6179 resulted in 2-3 log reductions at the same time. Growth/no growth interface of 6179 was slightly shifted, during storage, towards higher salt concentrations and lower pH values, than the respective interface for C_5.

Survival/no survival interface regarding acid challenge response was determined, in order to examine how suboptimal conditions and time of storage affect the response of both strains in low pH 2.0 (HCl).

Throughout storage, both interfaces regarding the two strains were identical. In addition, pH 5.5-6.4 and NaCl 2-4 % w/V resulted in higher survival rates during storage. On the other hand low pH (4.8-5.0) and high NaCl concentrations (8-10% w/V) resulted, for both strains, in no survival against subsequent acid treatment throughout storage.

Subsequent molecular approach of *Listeria monocytogenes* physiological response during storage and acid challenge showed that genes involved in acid and salt stress response differed in their activation/ deactivation point and the derived data were used for the construction of a transcription activation/deactivation interface, that could offer additional information of pathogen's response mechanisms.

CONCLUSIONS AND IMPACT OF THE STUDY

Our findings demonstrate that *Listeria monocytogenes* strains could differ in their growth and/or survival limits, regarding pH, NaCl presence and storage temperature, and the resulted interface is significantly affected by storage time. The results of gene activation/ deactivation interface describe the physiology of *Listeria monocytogenes* in probability terms and combined with survival phenotypes could be utilized for accurate stochastic assessment of the virulence potential of the organism.

Implementation of an individual-based model for microbial colony dynamics

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OBJECTIVE(S)

Traditionally, mathematical models in predictive microbiology are set up as low-complexity systems of coupled differential and/or algebraic equations that express the dynamics of macroscopic characteristics of the integral microbial population (e.g., global cell density). These models are accurately applicable to describe the planktonic dynamics of pure cultures with a relatively high cell number in homogeneous liquids. For this reason and because of their low complexity, macroscopic models are frequently applied in food industry. However, most food products consist of a structured medium that restricts microbial mobility. According to this restriction, microorganisms grow locally in dense *colonies*. The relatively high cell number in these colonies causes nutrient overconsumption and overproduction of acid metabolites. Consequently, nutrient depletion and acidification occur in the colony center. This implies that the cells exhibit strongly *different individual behavior* according to their position along the colony radius. As a consequence, the traditional macroscopic models that consider the global dynamics of a population of similarly behaving organisms are not appropriate to predict microbial dynamics in structured media.

METHOD(S)

In order to take into account the different individual behavior of microorganisms in a colony, the colony dynamics are modeled by means of an *individual-based model*. In this individual-based model, the individual cell is considered as the modeling unit instead of the global population. This implies that the global colony dynamics are not modeled explicitly, but emerge from sub-processes at the microscopic level.
These sub-processes include (*i*) the individual behavior of the cells (i.e., growth, reproduction and survival in stressful conditions), (*ii*) interaction of the microorganisms with the medium (i.e., substrate uptake and metabolite excretion), (*iii*) mutual interactions between cells (i.e., avoiding spatial overlap) and (*iv*) transport processes in the medium (i.e., diffusion of nutrients and metabolites).
As a case study, colony dynamics of *Escherichia coli* K-12 MG1655 in BHI-impregnated gelatin are considered. During the last decade, many experimental data about this *E. coli* strain have been obtained at BioTeC. Several software toolkits provide an easy-to-use framework to implement the individual-based model. As mature colonies contain millions of cells, special attention is paid to simulation run times. For this reason, the Repast Simphony software toolkit is selected. In addition, only a two-dimensional layer of cells is simulated. Diffusion processes are modeled by means of an ADI scheme that is implemented with the computationally efficient Thomas algorithm.

RESULTS

From the simulations with the individual-based model, typical colony behavior emerges, e.g., a linear increase of the colony radius in case of diffusion-limited colony growth[1]. Varying the diffusion coefficient or initial concentration of the nutrient implies changes in the colony morphology, which is in accordance to previously obtained experimental data[2] and simulations[3]. Due to nutrient deprivation and acidification of the colony center, a central zone of inactivated cells emerges.

CONCLUSIONS AND IMPACT OF THE STUDY

Traditionally, models in predictive microbiology describe macroscopic characteristics of an integral microbial population with similarly behaving microorganisms in homogeneous liquid media. In order to relieve the inaccuracies of these models in predicting microbial dynamics in structured food media, an *individual-based model* has been implemented in the easy-to-use software framework Repast Simphony. On the long term, the information that will be generated with the individual-based model will be incorporated in the mathematical structure of the classical macroscopic predictive models.

In this way, a low-complexity but more accurate model suitable for industry purposes will be created to describe microbial population dynamics in structured food media.

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Individual-based modeling or population approach for prediction of foodborne pathogens growth?

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OBJECTIVE

In this study, we investigated different approaches to describe the growth of *Listeria monocytogenes* in food, *i.e.* an individual-based modeling (IBM) approach coupled with a description of the micro-environment surrounding bacterial cells and a more classical population growth approach combined with a macro-description of the food physico-chemical characteristics. Two food matrices were used: a greatly variable matrix represented by smear soft cheese during ripening and a less variable food exemplified by frozen cold-smoked salmon.

METHODS

The characteristics (pH, aw) of smear soft cheese and cold-smoked salmon were measured with micro-methods, *i.e.* pH micro-electrode with a 50 µm tip diameter (Unisense) and osmolality of 100 mg-samples measured with a cryoscopic micro-osmometer (Osmomat). The macro-scale characteristics were measured with a conventional Hanna pH-meter and a GBX FA-st/1 water activity meter. Models were proposed to describe the variability of the environment with time and on the food surfaces. These models were combined with predictive microbiology models (Baranyi and Roberts, 1994; Augustin et al., 2005) describing the growth of *L. monocytogenes* with a deterministic population approach or by taking into account the single cell growth probability and lag times (Augustin and Czarnecka-Kwasiborski, 2012). Validation studies performed with irradiated cheese and salmon showed that the IBM approach combined with the micro-scale description of food environment was more relevant that the conventional population approach when the number of contaminating cells is low but predictions were closer when this number increased.

Simulations performed in this study aimed at assessing differences obtained when predicting the growth of a few cells of *L. monocytogenes* (10 cells) contaminating cheese and salmon surfaces after 20 days of ripening at 13.5°C and 15 days of storage at 8°C, respectively. The impact of the within-and between-batch variability of food characteristics was particularly assessed.

RESULTS

Table 1 presents results obtained with the two approaches. Contrary to the population approach, the IBM was characterized by its ability to predict no growth. For the population approach, this problem could be circumvented by calculating the single cell growth probability corresponding to mean properties of food. For instance, for salmon with pH 5.94 and aw 0.962, the *L. monocytogenes* growth probability at 8°C is 16% for one cell, thus the no growth probability for 10 cells is approximately equal to 18%. In spite of this improvement, the variability of the bacterial contamination simulated with the population/macro-scale approach was always smaller than the variability generated with the IBM/micro-scale approach. This was the case even with salmon exhibiting a low physico-chemical variability or when considering large sources of variability with a between-batch approach where we could think that this variability would have minimized the significance of the micro-scale approach.

Table 1. *L. monocytogenes* concentrations on smear soft cheese after 20 days of ripening at 13.5°C and on cold-smoked salmon stored at 8°C for 15 days initially contaminated with 10 cells predicted according different simulation approaches (IBM or population and micro- or macro-scale variability of food characteristics).

	Cheese				Salmon			
	IBM/micro		POP/macro		IBM/micro		POP/macro	
	wb[a]	bb[a]	wb	bb	wb	bb	wb	bb
No growth (%)	19%	27%	0%	0%	18%	18%	0%	0%
Mean[b] (log_{10} cfu/surface)	4.5	4.5	4.6	4.6	4.9	4.9	5.7	5.6
SD[b] (log_{10} cfu/surface)	0.70	0.77	0.36	0.54	0.40	0.63	0.15	0.21

[a] wb and bb, within- and between-batch variability; [b] mean and standard deviation when growth occurs.

CONCLUSIONS AND IMPACT OF THE STUDY

The proposed modeling framework combining IBM and description of the food micro-environment seemed more suitable than the conventional population approach to accurately assess the behavior of bacteria contaminating foods with a few cells and predict risky situations but also no growth situations.

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Modelling transfer of Escherichia coli O157:H7 and Listeria monocytogenes during preparation of fresh-cut salads: impact of cutting and shredding practices

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OBJECTIVES

Fresh cut salads are potential vehicles of pathogens due to pre-harvest contamination, or cross-contamination during preparation. Model simulating bacterial transfer between cutting equipment and fresh produce is of high value for quantitative microbiological risk assessment of fresh cut salads. Our objectives were: i) to define the distribution of *Escherichia coli* O157:H7 and *Listeria monocytogenes* transfer rates between cutting knives and lettuce leaves and ii) to model the bacterial transfer from knives to fresh cut salads and *vice versa* during consecutive cuts of leafy greens, simulating preparation of fresh cut salads, also involving the sporadic introduction of contaminated leaves.

METHODS

Three-strain composites of *E. coli* O157:H7 and *L. monocytogenes* (serovars 1/2a, 4b) were used to dip inoculate fresh water-washed lettuce leaves with a population of 10^5 (HI) or 10^3 log CFU/g (LI). Following attachment of pathogens (1h, 4oC), the lettuce leaves were centrifuged to remove excessive inoculum. A hundred sterile knives were used to create single cuts of the inoculated (HI) leaves (one knife per cut). The same procedure was applied for inoculated lettuce leaves after 4 days at 4°C. Level of pathogens was enumerated on knives by swabbing. The % transfer to knife was determined as "cfu on knife/cfu on contaminated lettuce". Next, the extent of transfer from knife to lettuce during consecutive cuts was evaluated. A sterile knife was contaminated by cutting inoculated lettuce leaves (bearing HI or LI levels). Subsequently, the knife was used to perform 30(HI) or 15(LI) consecutive cuts of noninoculated leaves. Leaf samples (10 g) were withdrawn during cutting for enumeration of pathogens remaining on knife or transferred to lettuce. The model suggested by *Møller et al.* (2011) to predict transfer (log CFU/g) of *Salmonella* Typhimurium DT104 during the grinding of pork was used to describe the transfer of *E. coli* O157:H7 and *L. monocytogenes* during cutting of fresh uncontaminated lettuce with contaminated knife. The model performance was evaluated as follows: contaminated batches of leaves (HI or LI) were 'introduced' at specific points of the cutting process and the model simulated the transfer of the two organisms on knives and on each of the cut leaf sample. The model was also used to simulate the transfer of the two pathogens during knife-cutting of cabbage and spinach, as well as during shredding of cabbage with a household vegetable shredder (extrapolation experiments).

RESULTS

The transfer percentage of *E. coli* O157:H7 from contaminated lettuce to uncontaminated knives during independent cuts varied from 0.1 to 53.01%. For *L. monocytogenes*, the respective percentage ranged from 0.20 to 18.16% on the first day and increased for the 4-days stored leaves up to 79.18%. For both pathogens the distribution was left-skewed . Regarding consecutive cuts, a rapid initial transfer was followed by an asymptotic tail at low populations moving to lettuce or residing on knife. Although the same pattern was observed for the two microorganisms, *E. coli* O157:H7 was transferred at slower rates compared to *L. monocytogenes.* These trends were sufficiently described by the transfer model, showing low RMSE values of 0.799-0.907 and 0.426-0.613 for *E. coli* O157:H7 and *L. monocytogenes*, respectively. The model also

showed good performance in validation trials with 5/0.8 -1.5, and *Af* 1.2-1.5. However, using the electric shredder to cut cabbage, the predicted trends were similar, but the model tended to underestimate the transfer of bacteria. This is probably associated with the accumulation of contaminated vegetable residues in the shredder. The model also slightly underestimated bacterial transfer during knife-cutting of cabbage and spinach, possibly due to differences in the microstructure of vegetables that might affect bacterial adhesion.

CONCLUSIONS AND IMPACT OF THE STUDY

The present model could be a useful tool for the assessment of risk during preparation of salads made of leafy greens, predicting the impact of potential cross contamination scenarios on product safety.

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A Neural Network Approach for Food Sensory Quality Prediction

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OBJECTIVES

Our long-term objective is to develop a decision support system (web-based software tool) that will allow the high value food industry for prediction of product safety, quality and shelf life of ready-to-eat products. It includes the study, simulation and optimisation of a large number of variables like raw material selection, product formulation, different processing steps, effect of each production step on the safety, shelf life, as well as considering quality under real conditions.
The main objective of this exploratory work is to apply artificial neural network (ANN) approach to evaluate its capability in predicting of food sensory quality, namely, appearance, odour, and texture. Experimental validation will be conducted to ascertain whether the developed method could yield models showing improved prediction of sensory quality in food.

METHODS

In the current work, iceberg lettuce samples are selected for the model data generation. Pre-made OPP film bags (Amcor Flexibles P-Plus, Bristol) are used for all the treatments used in the packing of iceberg lettuces samples. After storage for 2, 4, 7, 9, 11 days, sensory quality of each packed sample was evaluated by a panel of experienced sensory assessors. The sensory attributes of appearance, odour and texture will be evaluated using specific graded specifications for each separate fresh prepared product item.

ANN is not new in concept, but research interest in food area has increased significantly in the last decade. The major reason for this interest is the generic nature, approximation capabilities, and adaptability of ANN models. ANN provides a useful and effective tool for modeling the complex and poorly understood processes that occur in nature, as they are able to extract functional relationships between model inputs and outputs from data without requiring explicit consideration of the actual data generating process.

In the present study, a multilayer feed-forward neural network (MFNN) model was implemented in Matlab (version 2012a) and was utilized to correlate three output parameters (appearance, odour, and texture) to the inputs parameter, namely, temperature, gas composition, and storage time. The back propagation (BP) algorithm, the most popular neural network learning method, was implemented for training, testing and validation. Data will be selected randomly, 20% of data testing, 20% of data for validation, and the rest of data for training. In this study, two hidden layers were found to be appropriate to develop the model. However, the number of hidden layers and neurons within each hidden layer can be varied based on the complexity of the problem and data set. To determine the optimal network configuration, the effects of uncertainties in output experimental data and ANN prediction values on root mean square error (RMSE) were studied.

RESULTS

Mode sensitivity was evaluated for several different networks with 1, 5, 10, 15, 20, 25, 30 nodes in the first hidden layer and 5, 10, 15, 20, 25, 30 nodes in the second layer, correspondingly. Increase in the number of neurons in each layer did not lead to any improvement in the results. Weights and biases were adjusted automatically by the back-propagation training algorithm. The training process stopped when algorithms

truly converged which means that performance/error goal was achieved. The prediction performance was tested using a new data set. The network with a 4-15-25-3 was found to have the best performance.

The qualitative comparison of the sensory quality curves as predicted by the ANN model versus the results determined experimentally is presented. The associated RMSE values with the optimal ANN model for appearance, odour and texture are 0.38, 0.32 and 0.53, indicating a very good agreement between the experimental and ANN model predicted values.

CONCLUSIONS

In this study, an artificial neural network modeling approach was developed and tested for predicting the experimental sensory quality of iceberg lettuce during storage. The ANN model presented can easily be used without any elaborate programming. The best ANN configuration of predicting sensory quality included 2 hidden layers with 15 neurons in the first hidden layer and 25 neurons in the second layer. A reasonable very low-level value of the estimated error was an indication that the network prediction was still supported even though the experimental data were not sufficient in number. The procedure proposed can also help the researchers to plan new measurements by showing where these are needed.

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Modelling the rate of transfer of antibiotic resistance between E coli strains cultured under well controlled environmental conditions.

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OBJECTIVE

In quantitative microbiological risk assessment (QMRA) the contamination level of all relevant pathogens of raw materials is considered, but resistance to antibiotics, caused by the abundant use of antibiotics in animal husbandry, is often ignored. The principle concern is that the use of antibiotics in food animals will select for resistance to these agents in zoonotic intestinal bacteria and, via food-borne transmission an infection in human beings will develop that is untreatable (Van et al., 2007, 2008). Schuurmans et al. (2011) showed in continuous cultures that transfer of tetracycline resistance could occur when the fermenters were inoculated with one tetracycline sensitive and one resistant strain of *E. coli*. However, a quantitative estimate of the rate of transfer has not yet been made.

METHODS

From data of specific glucose consumption (Schuurmans et al., 2010) we could infer the growth rate of the tetracycline sensitive acceptor. To estimate the relationship between the mutation rate and the appearance of demonstrable numbers of mutants we developed a deterministic model that predicts the fate of both strains in the fermenter. The growth rate of the donor strain remained equal to the dilution rate until a substantial number of resistant acceptor cells had been formed whereas the growth rate of the acceptor strain remained 5 – 10 % higher under these conditions. In the end tetracycline resistant acceptor cells increase gradually in the fermenter and eventually they overgrow the donors. From the time to detection of resistant acceptor cells the number of gene transfer events resulting in tetracycline resistant acceptor cells could be deduced. The detection level for the presence of cells from the recipient strain was about 3200 cells per fermenter. To predict varability also a stochastic model was developed based on based on the probability of growth and wash out predicted by Monte Carlo simulations.

RESULTS

The deterministic model could predict the average expectation of number of resistant acceptor cells dependent on frequency of gene transfer and incubation time. The model shows quantitatively that the growth rates of both strains are apparently stable until a very large number of the acceptor strain is formed ($> 10^{10}$ cells per fermenter). Whereas the deterministic model gives a good estimate of the expected development of the resistant acceptor strain, a large variation in the 'population' of possible results may occur, in particular when a low initial number of 'mutant' resistant acceptor cells are formed. Hence, in addition to the deterministic model, the stochastic model was developed. As no analytical solution was available, a Monte Carlo approach was followed. The mean number of resistant strains dependent on 'mutation rate' by the deterministic model agreed well with the prediction by the stochastic model. Interestingly, the stochastic model could also predict the *probability* of detecting gene transfer events.

CONCLUSIONS AND IMPACT OF THE STUDY

With continuous fermentation a quantitative estimate of the risk of transfer of antibiotic resistance could be made. Comparing the observation of Schuurmans et al (2010) and the model predicting the probability of the formation of tetracycline resistant acceptor strain cells during fermentation, strongly suggests that the gene transfer event must have occurred several times. The early appearance of observed mutants cells suggest the early formation of many mutants in an early stage of culture.

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From individual cell "time-to-death" to statistical population dynamics of microbial inactivation

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OBJECTIVE(S)

Traditional approaches to microbial inactivation are based on deterministic mathematical models which describe the decline of microbial populations as a whole without considering the individual cells. However, the heterogeneity in the resistance of individual cells to a lethal stress (Cerf, 1977;Casolari, 1988) can be a significant source of variability in microbial inactivation during food processing. In most cases where large microbial populations are studied, variability is masked through the law of large numbers, and the system seems to behave in a deterministic manner even though the underlying law is stochastic. Food processing however, aims to completely eliminate pathogen contamination and the tailing of survival curves at the level of few cells is extremely important. The objective of the present study was to use a statistical approach based on individual cell "time-to-death" (TTD) probability distributions in order to study and quantify variability in microbial inactivation.

METHODS

The inactivation kinetics of non-acid adapted and acid adapted Salmonella enterica serotype Agona (FSL S5-867) as single or mixed cultures were studied at 25°C under acidic conditions both at population and single cell level. A 18-h culture of *S. agona* was added to TSB-G acidified to pH 3.5 with lactic acid. Various initial concentrations from 10^9 to $10^{2.5}$ cfu/ml were tested. Single cell inactivation was monitored using time lapse confocal scanning laser microscopy. Stained cells of S. Agona with the fluorescent dye SYTO 9 (green) were inoculated to TSA covered with acidified water (pH 3.5) and the red dye propidium iodide. A sequence of frames for the same field of view with time were obtained and allowed for the discrimination between dead and alive cells (red/green).

RESULTS

The survival data were transformed to $(N_0-N_t)/N_0$ vs time which corresponds to the cumulative probability distribution of the TTD of the individual cells in the population. For a high N_0 (10^9 cfu/ml) we can assume that this distribution approaches the true distribution of individual cell "time-to-death". After fitting the observed data for $N_0=10^9$ to various distributions, the three best fitted distributions based on the RMSE criterion were used to predict inactivation for various N_0 and quantify the variability. Predictions were based on Monte Carlo simulations with the number of iterations and simulations representing the N_0 and the variability in the number of survivors, respectively. The results showed that variability is negligible when the number of survivors is higher than 100 cells but increase significantly at lower levels. In addition variability is significantly affected by the N_0. The %CV of the time for a total population reduction of non acid adapted cells increased from 10.7% for $N_0=10^5$ to 26.3% and 55.9% for $N_0=10^2$ and $N_0=10^1$, respectively. The results explained the variability observed in the experiments with low initial concentrations N_0 ($10^{3.5}$ and $10^{2.5}$ cfu/ml). Simulations on microbial inactivation of a mixed population with acid and non-acid adapted cells were also performed by combining the respective distributions of individual cell TTD. Furthermore, the distributions estimated with plate count were validated with TTD data for individual cells obtained microscopically.

CONCLUSIONS AND IMPACT OF THE STUDY

The statistical approach proposed in this study allows for quantifying the variability in microbial inactivation. It can be used for the development of a risk-based process control system and improve the accuracy of risk assessment studies.

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A novel Poisson-gamma modelling framework to perform inferential statistics on microorganisms of low recovery in foods

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OBJECTIVE(S)

The Poisson-gamma distribution has been previously shown to be able to represent low microbial counts more efficiently than the normal distribution (Gonzales-Barron et al., 2011). For the conduction of inferential statistics on this type of microbial data, the common analysis of variance based on normality is not a reliable approach. This study introduces an alternative modelling framework for low microbial counts using a Poisson-gamma regression. As an illustration, the effect of chilling on the concentration of total coliforms from beef carcasses was determined applying the new approach.

METHODS

Plate count data of total coliforms were obtained from beef carcasses (n=600) sampled in a total of twenty visits to eight large Irish abattoirs. Each of the carcasses was swabbed before and after chilling. The sampling process was represented by a Poisson distribution while the unknown microbial concentration was modelled by a gamma distribution with parameters mean (λ) and dispersion (k). The Poisson-gamma (PG) regression model with two random-effects placed in λ and k accounting for between-batch variability was compared against a zero-modified hurdle Poisson-gamma (HPG) regression with three random-effects in λ, k and in the within-batch probability of zero counts (ω_0). The algebraic expressions for every parameter consisted of an intercept and a slope (i.e., coded covariate) to quantify the effect of chilling.

RESULTS

The HPG model (BIC=10683) fitted the coliforms data better than the PG model (BIC=10817). However while their performance for the batches of pre-chill carcasses was comparable, it was mostly for the batches of post-chill carcasses that the superiority of the HPG was noticeable, as evidenced by its better capacity to predict the proportion of zero counts. In terms of model validation, the HPG model predicted better the post-chill distributions from the pre-chill observations in all five test batches. This occurs because the HPG is mathematically equipped to deal with higher proportion of zero counts (higher variance) than the PG model. Extracting the significant between-batch variability in the coliforms mean concentration σ^2_u=2.68, in the dispersion measure σ^2_v=2.39 and in the probability of zero counts σ^2_w=0.89, the HPG model proved that the chilling operation decreases significantly the viability of the total coliforms, and the pre-chill to post-chill counts reduction in a batch is by an average factor of 2.2 (95% CI: 2.15 – 2.24). The model also indicated that chilling increased the expected probability of zero counts from 7% to 49%, and that the higher the coliforms concentration (i.e., the poorer the hygiene), the weaker the effect that chilling has on the beef carcasses to reduce such contamination.

CONCLUSIONS AND IMPACT OF THE STUDY

Although the common data analysis based on normality constitutes a more straightforward procedure, special care should be taken as it cannot be used in all cases, certainly not when microbial counts have many non-detections. In such cases, the Poisson-gamma models offer a suitable framework to perform inferential statistics, which offer many advantages such as: (i) it takes in the zero counts in a natural way so there is no

need to replace them with limit of quantification, nor fitting censored normal distributions without the certainty that the distribution is actually normal; (ii) it can represent equally well batches that have occasionally higher microbial concentrations; (iii) it produces estimates of concentration in arithmetic mean, and not in geometric mean; (iv) the zero-inflated variant, although not demonstrated in this study, has the capability to distinguish between true absence of contamination and false non-detections due to sampling.

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Prediction of coculture growth kinetics between Bacteroides thetaiotaomicron and Bifidobacterium based on monoculture growth kinetics

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OBJECTIVES

Commensal intestinal bacteria have been found to have a significant effect on human health primarily by fermenting non-digested food components. Short chain fatty acids (SCFA) produced as a result of fermentation (mainly polysaccharides and resistant starch) provide an energy source for the intestinal epithelium, heart, brain and skeletal muscles. The purposes of this study are:

- To develop mathematical models of the growth kinetics of *Bacteroides thetaiotaomicron* and 18 *Bifidobacterium* strains grown in monoculture on three different substrates (fructose, oligofructose and inulin) along with the simultaneous or preferential degradation of the substrate fractions and the production of metabolites (acetate, succinate, formate, lactate).

- To develop a model of the cross-feeding between *Bacteroides thetaiotaomicron* and *Bifidobacterium* in coculture and determine the ability of the model to predict the coculture kinetics (bacterial growth, substrate degradation, and SCFA production) based on the monoculture kinetics.

METHODS

We modified the mechanistic model of Amaretti et al. (2007) and fit the model to the multi-response data (bacteria, substrate, and metabolite concentrations) reported by Falony (2009) and Falony et al (2009 a,b) using nonlinear regression (Bates and Watts 2007) to determine model parameters. The experimental data were from monoculture experiments of the growth kinetics of *Bacteroides thetaiotaomicron* LMG 11262 and 10 *Bifidobacterium* species (constituting 18 strains) for three different substrates (fructose, oligofructose and inulin) (Falony 2009; Falony et al. 2009a; Falony et al. 2009b). We also developed a model to describe microbial cross-feeding between two bacterial populations. This model assumes that one bacterial population releases fragments of polysaccharides as a result of extracellular degradation of the substrate and that the other bacterial population utilizes the fragments for growth. We used the parameter values determined by fitting our model to the monoculture experiments to predict the measured coculture dynamics between *B. thetaiotaomicron* LMG 11262 and *Bifidobacterium longum* LMG 11047 and *B. thetaiotaomicron* LMG 11262 and *Bifidobacterium breve* Yakult.

RESULTS

We found that the model adequately described the multi-response mono-culture data (bacteria, substrate and metabolite concentrations (acetate, succinate, formate, lactate)) for inulin and fructose (cumulative R^2 =0.99, 0.99). Oligofructose degradation appeared to occur with two phenotypes: simultaneous degradation of oligofructose fractions or preferential degradation, where the bacteria degraded oligofructose chains in order of low degree of polymerization to high, or vice versa. The modified model that include preferential degradation was able to describe the multi-response mono-culture data for simultaneous degradation (13

bifidobacteria strains) and preferential degradation (5 strains) of oligofructose (R^2 =0.99, 0.99, respectively). Using our cross-feeding model we found that the coculture growth kinetics of *B. thetaiotaomicron* LMG 11262 and *Bifidobacterium longum* LMG 11047 were accurately predicted using the best fit monoculture parameters (R^2 =0.98). For the coculture growth kinetics of *B. thetaiotaomicron* LMG 11262 and *Bifidobacterium breve* Yakult, the prediction based on mono-culture kinetics was adequate provided there was a decrease in the specific growth rate of B. thetaiotaomicron LMG 11262 (R^2 =0.97).

CONCLUSION AND IMPACT OF THE STUDY

The model developed is able to describe the growth of *Bacteroides thetaiotaomicron* LMG 11262 and 18 *Bifidobacterium* strains in mono-culture along with substrate utilization for three different substrates (fructose, oligofructose and inulin) and metabolite production (acetate, succinate, formate, lactate). The model was also able to describe the coculture dynamics between *B. thetaiotaomicron* LMG 11262 and *Bifidobacterium longum* LMG 11047 and *B. thetaiotaomicron* LMG 11262 and *Bifidobacterium breve* Yakult. Of particular importance is that the parameters derived from monoculture experiments could be used to predict the coculture dynamics. Multi-culture bacterial dynamics may be able to be described based on selected mono-culture experiments.

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Finding a tradeoff between a microbiological risk and a nutritional benefit in foods using a Quantitative Microbial Risk Assessment approach

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OBJECTIVE(S)

Heat processes applied to foods are based on a tradeoff between microbiological safety or stability, and quality (organoleptic characteristics, nutritional value…) of the processed food. The objective of this work was to propose an approach to optimize of the canning process of green beans in accounting for both non stability due to survival and growth of the thermophilic spore-forming bacteria *Geobacillus stearothermophilus* (the microbiological risk) and the vitamin C concentration (the nutritional benefit), an important nutrient in fruits and vegetables, and often used as a marker of process impact on the nutritional value of foods.

METHOD(S)

A model predicting simultaneously the risk of microbiological non-stability at 55°C (a requirement in the canning industry) of canned green beans due to *G. stearothermophilus*, and vitamin C degradation along green bean canning process and storage was built and validated with independent sets of data, such as % of no stability observed in industry or measurement of vitamin C concentrations (Rigaux, 2013). In particular, a vitamin C degradation time at 85°C and at 80 $kJ.mol^{-1}$ DHA activation energy named F_1 and the microbiological thermal death time at 121°C and at reference value z_T = 10°C (F_0) were introduced in the model to allow a simultaneous prediction of vitamin C degradation and bacteria inactivation for any given time and temperature during the sterilization process. Parameters of the model were fixed, variable, uncertain, or both uncertain and variable. Uncertainty and variability on parameters were separately propagated through the model within the two-dimensional Monte-Carlo simulation framework.

RESULTS

A mean risk of non-stability due to *G. stearothermophilus* of 0.5% of green bean cans (corresponding to usual industrial observations) was estimated with a 95% uncertainty interval of [0.1%, 1.2%], as the % of cans containing more than 12 mg vitamin C/100 g (i.e. exceeding the nutrition claim limit) was estimated at 3.2 % in mean with a 95% uncertainty interval of [0.1%, 13.2%]. As the mean initial concentration was 17.0 mg vitamin C/100 g with a 95% variability interval of [4.0, 30.6], the model reports a significant degradation of vitamin C. Moreover the model predicts a relative independence between vitamin C concentration and *G. stearothermophilus* concentrations in canned green beans after sterilization. This offers the possibility of optimizing process parameters influencing one output without affecting the other. Then the model was used to test the consequences of some alternative process scenarios maintaining an acceptable level of microbiological non-stability, while increasing the vitamin C concentration. For instance reducing blanching time, increasing F_0, reducing waiting time before blanching and slightly acidifying the covering brine predicted simultaneously a % of non-stability decreased to 0.1% in mean with a 95% uncertainty interval of [0.0%, 0.7%] and more than 30% cans containing concentrations in vitamin C greater than 12 mg/100 g (33.5% in mean with a 95% uncertainty interval of [7.8%, 52.5%].

CONCLUSIONS AND IMPACT OF THE STUDY

Finding a tradeoff between risk and benefit, between food safety and quality, is a common issue in food industry. The modeling framework proposed in the present work, which considers variability and uncertainty of the modeled phenomena, is increasingly familiar to scientists developing Quantitative Microbial Risk Assessment in food. We showed this framework is also adapted to model the evolution of nutrients in foods: parameters of chemical reactions, initial concentrations in nutrients for instance are also uncertain and/or variable. We could therefore consider that food microbiology predictive and probabilistic approaches are useful tools for the design of safe food processing and improvement of food quality looking at the joint distribution of the two criteria.

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Predator-prey type mathematical model explains novel mode of interaction between Salmonella and gut bacteria

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OBJECTIVES

It has been reported that gut bacteria from healthy mice effectively outcompete *Salmonella enterica* subsp. *enterica* serovar Typhimurium (Ahmer & Gunn, 2011). The aim of this work was to investigate if bacteria obtained from human faeces could have the same effect on the population dynamics of *S.* Typhimurium.

METHODS

An experimental colon model was used in order to replicate the conditions of the human colon. The cultures were magnetically stirred and heated at 37^{o}C by a Grant (Jencons-Pls, UK) circulating water bath. The pH of the medium was maintained between 6.8 and 7.2 by a pH control Fermac 260 (Electrolab, UK). The medium was continuously sparged with O_2-free N_2 to maintain anoxic conditions.

RESULTS

A decrease of ca. 10^4-10^5 CFU/ml of *S.* Typhimurium population was observed in batch anaerobic cocultures with faecal samples from different human donors. The loss of culturability of *S.* Typhimurium population required cell-cell contact with faecal bacteria. This effect was not observed when a 0.45 µm pore size filter separated *S.* Typhimurium from the faecal bacteria, when *S.* Typhimurium was inoculated into supernatant obtained from feacal cultures or when in contact when faecal cells that had been inactivated.

A mathematical model of a predator-prey nature explains this phenomenon. The model describes the inactivation of *Salmonella* as the result of the frequency of encounters between bacterial cells and the probability of inactivation after encounter. The frequency of encounters depends on the concentrations of both populations. The expression $F\ (S{\cdot}F)^{1/2}$ quantifies the concentration of both populations, faecal bacteria, *F*, and *S*. Typhimurium, *S*, required to observe inactivation of *Salmonella.* When the value of $F\ (S{\cdot}F)^{1/2}$ decreased below ~ 10^{15} (cfu/ml) ^{2}, encounters between cells were not sufficient to observe significant inactivation. The probability of inactivation after encounter was constant with an estimated value of ~10^{-5} and it is characteristic of the mechanism of inactivation after encounter.

CONCLUSIONS AND IMPACT OF THE STUDY

Most of the identified pathogenic bacteria exclusion mechanisms associated with the gut microbiota are mediated by extracellular products (Ashida, Ogawa, Kim, Mimuro, & Sasakawa, 2012; Keeney & Finlay, 2011). A novel way of interaction between commensal gut microbiota and *S.* Typhimurium that requires cell contact and leads to growth inhibition of *S.* Typhimurium is demonstrated by this work. The model presented explains the observed kinetics of the loss of culturability of *S.* Typhimurium as a function of the frequency of encounters between the two populations and the probability of inactivation after an encounter. This new model will now be useful for applying the finding of this study to ongoing efforts to reduce *Salmonella* infection.

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ADVANCES IN METHODS AND MODELS IN PREDICTIVE MICROBIOLOGY

Posters

Probabilistic modeling of Bacillus sp. spore lag time applied to the sterility testing of UHT milk

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OBJECTIVE(S)

In association with Monte Carlo simulations, predictive microbiology models allow to simulate and quantify rare events that may not be evidenced through laboratory testing. Taking into account the lag time when predicting the growth of spore-formers is often crucial because the lag time may represent a significant part of the time to growth to an unacceptable level, or the time to detection. The lag time varies as a function of the strain, the conditions of sporulation, the stresses the population of spores was subjected to, and the environmental conditions of outgrowth. On top of that, the germination process seems to display a stochastic pattern, leading to between-cell variability.

The purpose of our study was to model the outgrowth of a heat-stressed spore of a *Bacillus* sp. strain in milk. This model was intended to estimate the time needed for the pre-incubation of UHT milk in the context of sterility testing, assuming that a unit may be contaminated by as little as 1 spore.

METHODS(S)

A strain of *Bacillus* sp. was selected and, for the purpose of the study, spores cultures were subjected to a heat stress of 101°C for 30 seconds. The optimal growth rate in milk was estimated by challenge-testing. Individual lag times were measured in BHI by Bioscreen^{TM}.

RESULTS

The optimal growth rate in UHT milk was estimated at 2.3 h^{-1}. Individual lag times in BHI at 35°C (146 values) ranged from 0.5h to 32h with a median value of 2.8h. Results were combined with other data from the literature to develop a stochastic model intended to predict the outgrowth of a heat-stressed spore of *Bacillus* sp. in milk.

CONCLUSIONS AND IMPACT OF THE STUDY

We confirm the stochastic pattern of the germination process of heat-stressed spores of *Bacillus* sp. in milk. Probabilistic modeling helps to predict the outgrowth of spores in the context of low to very low initial contamination. Such predictive model will help quality managers to establish the time needed for the pre-incubation of UHT milk in the context of sterility testing.

A binary logistic regression model for classification of mechanically separated meat

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OBJECTIVE

Mechanically separated méat (MSM) is defined in the current EU Régulation (EC) No 853/2004 as the product obtained by removing méat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure; two types of MSM products (low and high pressure) are defined according to the altération of bone structure and calcium content. Différent interprétations of the définition of MSM hâve led some Member States to consider low-pressure MSM products as méat préparations. Therefore, the European Food Safety Authority (EFSA) was asked to issue a scientific opinion on the public health risks related to différent types of MSM and to sélect, rank and suggest objective measurement methods and values for parameters to classify MSM types.

METHODS

Concerning the parameters for the classification of MSM, a database on chemical characteristics of hand deboned méat and MSM from poultry and pork was developed by retrieving data from the literature (not systematically designed for the purpose of this analysis) on moisture, protein, fat, ash, calcium, iron, cholestérol and collagen. The chemical characteristics of hand-deboned méat and MSM were compared both graphically and statistically in order to sélect the most appropriate parameters.

RESULTS

The analysis of the available data showed statistically significant ((P<0.05) différence between méat and MSM for ail tested chemical characteristics, but, due to overlapping of data, the discriminatory power provided by protein, ash and iron contents was low, thus indicating that thèse characteristics are not good indicators for classifying a product as MSM. No significant (P>0.05) overlapping was observed for calcium and cholestérol indicating that thèse could be used as appropriate indicators for classifying a product as MSM. However, for the latter parameter the available data with MSM are limited and further research for validation is required. A binary logistic régression analysis was performed in order to identify the probability for a product to be classified as MSM based on calcium content. The data were fitted to a logistic régression model using Minitab software (Minitab Inc. PA, USA). The automatic variable sélection option with a stepwise sélection method was used to choose the most significant effects (P<0.05), and différent predicted interfaces were calculated. The model was incorporated into an Excel application where values for calcium content can be introduced and the probability for a product to be classified as MSM can be estimated. The analysis showed that calcium content of 21, 39 and 81.5 and 100 mg/100 g corresponded to probabilities of 0.1, 0.5, 0.9 and 0.936 for a product to be classified as MSM.

CONCLUSIONS AND IMPACT OF THE STUDY

The present model is a first step for the quantitative approach in the distinction between MSM types and of MSM from other méat products, based on objective data. However, until specifically designedstudies for validation become available, the outcome of this model cannot provide definitive conclusions on the differentiation between different types of MSM.

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Units of measure identification in unstructured scientific documents in microbial risk in food

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OBJECTIVE(S)

A preliminary step in microbial risk assessment in food is to gather and capitalize experimental data. Data capitalization is a crucial stake in an overall decision support system which consists of predicting microbial behavior [1]. In the framework of the French ANR project MAP'OPT (Equilibrium Gas Composition in Modified Atmosphere Packaging and Food Quality), the predictive modeling platform Sym'Previus (www.symprevius.org) should be able to propose a global approach to establish a scientifically sound method for choosing an appropriate modified atmosphere and associated packaging solution.

Our work is part of this overall system and aims at extracting semi-automatically experimental data from unstructured scientific documents. Indeed, these documents use natural language combined with domain-specific terminology that is extremely time-consuming and tedious to extract in the free form of text and therefore to gather and capitalize. Our work relies on the MAP'OPT-Onto ontology [4], which has been built as an extension of the ontology used in Sym'Previus by adding concepts about food packaging, quantity concepts and concepts managing units of measures.

Experimental data are often expressed with concepts (e.g *packaging*, *permeability*) or a numerical value often followed with its unit of measure (e.g. *258 amol m-1 s-1 Pa-1*). In this paper, our work deals with unit recognition, known as a scientific challenge.

METHOD(S)

Extracting automatically quantitative data is a painstaking process because units suffer from different ways of writing within documents. We can encounter same units written in different manners such as *amol* m^{-1} s^{-1} Pa^{-1} written as *amol*.m^{-1}.s^{-1}.Pa^{-1} or as *amol/m/s/Pa*. We aim at focusing on the extraction and identification of these variant units seen as synonyms, in order to enrich iteratively an ontology, which represents a predefined vocabulary used to annotate, capitalize and query experimental data extracted from texts [2]. Our work addresses unit extraction and identification issues from texts to enrich an ontology in a two-step approach. First, we use text-mining methods and supervised learning approaches in order to predict relevant parts of the text where synonyms of units or new units are. The second step of our method consists in extracting specific strings representing units in the segments of texts found in the previous step. The extracted candidates are compared to units already present in the ontology using a new edit measure based on Damerau-Levenshtein [3].

RESULTS

We have made experiments on 115 scientific documents (i.e. around 35 000 sentences) on food packaging. Each unit is recognized from a list of 211 units already defined in the MAP'OPT-Onto. Our learning algorithms predict that almost 5 000 sentences contain units. This prediction is correct for 95,5% of cases. In the second step, we have successfully extracted 38 terms as either synonyms or new units from sentences selected in the first step. So, we can propose 18% of enrichment of the pre-existing MAP'OPT-Onto.

CONCLUSIONS AND IMPACT OF THE STUDY

We propose a two-step approach to enrich an ontology with unit synonyms. Our approach addresses both issues: location and extraction of units. Future work should be defined in order to automatically populate the ontology with new concepts (e.g. food product or packaging names) and link the new units discovered.

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Strain effect on the heterogeneity of individual cell growth kinetics of Salmonella Typhimurium

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OBJECTIVE(S)

Phenotypic variability among single bacterial cells has been reported (Korobkova et al., 2004), attracting the attention and interest of researchers, particularly of those working in the field of predictive microbiology. Significant single-cell heterogeneity was demonstrated by the findings of a recent study of ours which, by utilizing a time-lapse microscopy method, allowed for the evaluation of the growth dynamics of microcolonies originating from single cells of *Salmonella enterica* (Koutsoumanis and Lianou, 2013). In continuation to the latter investigation, the objective of the present work was the characterization of the single-cell growth variability of different strains of *S. enterica* serotype Typhimurium, and the evaluation of the existence of a strain effect on such variability.

METHODS

Five *S.* Typhimurium strains were evaluated in the present study. Twenty microliters of 24-h cultures of the strains, after two 10-fold serial dilutions in Ringer's solution were added to 500 μl of tryptone soy agar solidified on a glass slide, and, after allowing for drying of the inoculum for 5 min, the inoculated agar was covered by a coverslip and sealed with silicone to avoid dehydration. The colonial growth of single cells was monitored at 25°C by phase-contrast time-lapse microscopy using a z-motorized microscope (Olympus BX61) equipped with a 100× objective (Olympus) and a high-resolution device camera (Olympus DP71) (Koutsoumanis and Lianou, 2013). The high quality of images allowed for monitoring the number of cells in each microcolony with time using the ImageProPlus image analysis software, and for the determination of the first, second and third division times (FDT, SDT and TDT, respectively). Furthermore, after cell counting, data were transformed to the respective growth curves (i.e., number of cells in each microcolony as a function of time), and the latter were fitted to the primary model of Baranyi and Roberts (1994) for the estimation of the single-cell growth kinetic parameters for each one of the tested strains.

RESULTS

Depending on the tested *S.* Typhimurium strain, the mean FDT ranged from 1.69 to 2.75 h, while the corresponding ranges for the mean SDT and TDT were determined to be 0.76 to 1.03 h and 0.39 to 0.65 h, respectively. The coefficient of variation of the division times of individual cells ranged from 33.8 to 43.3% for the FDT, from 43.9 to 62.4% for the SDT, and from 39.9 to 57.7% for the TDT. The analysis of variance showed significant ($P<0.05$) differences among the 5 strains for the means of all three DT. Using the Levene's test and the Bonferroni confidence intervals for standard deviations significant ($P<0.05$) differences in the variance of individual cell DTs among strains were also found. The observed distributions of the kinetic parameters (i.e., lag phase and maximum specific growth rate) of microcolonies originated from a single cell were used to demonstrate the impact of variability in modeling growth of microbial populations of different strains using Monte Carlo simulations.

CONCLUSIONS AND IMPACT OF THE STUDY

The present study showed that the variability of individual cells growth kinetics may differ among strains. The results provide useful quantitative information for incorporating strain variability and heterogeneity in individual cell behaviour in stochastic growth models and risk assessment studies.

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Monte Carlo simulation as a tool to quantify temperature effect on Sacharomyces cerevisiae Pulsed Electric Fields inactivation

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Fermentative yeasts, specifically *Saccharomyces cerevisiae,* are the most common spoilage agents in refrigerated citrus juices. To extend fruit juices shelf life and preserve its safety, Pulsed Electric Fields has emerged as good alternative to process pumpable products, specifically beverages, with suitable retention of food nutritious and quality factors. This non-thermal technology mimic pasteurization processes results with final temperature values remaining below 50 ºC.

In the present study, the effect of PEF inlet temperature, 10 and 32 ºC, was assessed in terms of *Saccharomyces cerevisiae* reduction effectiveness after [10-40] kV/cm electric field strength applied to a new orange juice-milk mixed beverage, with treatment times in the range [0-1900] µs. Orange juice-skim milk mixed beverage (pH =4.05; σ=0.291 S/m) was inoculated with the spoilage microorganism (10^7 cfu/ml), and subsequently processed by PEF at defined conditions (flow rate=60 ml/min; pulse wide=2.5 µs; 8 treatment chambers). Temperature of the product was registered by 4 thermocouples connected at the entrance and exit of the first and fourth pair of chambers. Obtained experimental data were accuracy fitted to Weibull model (adjusted-R^2 =0.978; RMSE=0.042). According to obtained results, microbial inactivation was clearly dependent on inlet temperature, being the higher the PEF inlet temperature, the higher the spoilage control for each applied PEF condition, remaining final temperature below 52±2.04ºC for all studied PEF treatments. Low energy treatments ([10-20] kV/cm) were majorly influenced by inlet temperature with significantly increased ($p < 0.05$) microbial reduction levels. Temperature variability for each studied inlet condition was registered as previously mentioned, and its effect on microbial kinetics was reflected on Weibull scale parameter *b*. Both, initial *S. cerevisiae* distribution load, defined by a triangular distribution function (*triang(5.73;6.22;6.23)*; and best fitted scale parameter (e.g. 10 kV/cm: (i) 10ºC: *BetaGeneral(0.29;0.27;0.13;0.17)*; (ii) 32 ºC: *Normal(0.86;0.05))* were considered as inputs of either the stochastic Weibul model built at 10 and 32 ºC inlet temperatures. By Monte Carlo simulation, final *S. cerevisiae* loads obtained after 10 kV/cm-900 µs PEF processing of the orange juice-milk mixed beverage were 23984 cfu/ml (Loglogistic(7615,3;15542;2,5389)) and 7480 cfu/ml (Weibull(2,3593;7203;RiskShift(1591,5))) at 95 % of iterations under 32 and 10 ºC inlet temperatures respectively.

According to obtained results, it can be conclude that 32 ºC inlet temperature reduced final *S. cerevisiae* load by [7-2] times more with respect to 10 ºC inlet temperature under the same PEF processing treatment conditions. Increasing inlet temperatures could have a great impact on final microbial reduction levels due to PEF treatments, specifically at lower treatment times, which means that effectiveness of low energy PEF treatments could be enhanced by means increasing inlet temperatures.

A gamma-type model to describe the effect of water activity, temperature and pH on the proliferation rate of three moulds isolated from spoiled bakery products

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OBJECTIVE(S)

Moulds are able to grow within a wide range of water activity (aw), temperature and pH; they are regularly involved in bakery product spoilage. This causes a great economic loss estimated to more than €200 million per year in bread industry (Legan, 1993).

The objective of this work was to quantify the effect of water activity (a_w), storage temperature and pH on mould spoilage. A predictive model based on the gamma concept was developed for *Eurotium repens*, *Aspergillus niger* and *Penicillium corylophilum*, three moulds isolated from spoiled bakery products. The model was developed through Bayesian inference.

METHODS(S)

Experimental data were generated on Malt Extract Agar media. A 5-level Latin square design (van Derlinden et al., 2013) with a_w (from 0.80 to 0.98), temperature (from 15 to 35 °C) and pH (from 3 to 7) for each of the three species, was set.

From these experiments, radial proliferation rates, ***p*** (in mm/day) were estimated by fitting the increase of colony radius (in mm) over the time (in days) to a two-phase linear model using R software.

After a square root transformation, ***p*** values versus a_w, temperature and pH were fitted to a gamma function (equation 1) and the cardinal values (min, opt and max) of these latter factors were estimated for each of the three strains.

$$\sqrt{\mu_K} = \sqrt{\mu_K^{opt}} \cdot \sqrt{\left(\gamma_K(a_w) \cdot \gamma_K(T) \cdot \gamma_K(pH)\right)} + \varepsilon \qquad (1)$$

In Equation 1, *K* represents the strains, ***p*** the radial proliferation rate (mm/day), p^{opt} the value of ***p*** at optimal a_w, temperature (T) and pH. The ***y*** terms represent the effect of aw, temperature and pH on ***p*** and ***e*** the residual error of the model.

The estimation procedure was done using Bayesian inference with WinBUGS software, the prior distributions were defined according to the literature information. The model was a hierarchical one, considering the same residual error whatever the mould species. To assess whether the factors a_w, temperature and pH were significant, a model/submodel procedure was performed using both accuracy (standard deviation of residual error, $\mathbf{a_E}$) and parsimony as criteria.

RESULTS

The factors a_w and temperature had a significant impact on ***p*** for the three strains, while the effect of the pH

was not found significant for any of the three moulds (model with pH gamma term: $\mathbf{a_e}$ 0.010, number of parameters: 25; submodel without pH gamma term: $\mathbf{a_E}$ 0.011, number of parameters: 19). The final model chosen was therefore a gamma function describing only the a_w and the temperature effect. The same gamma term structure was used to describe the effect of temperature on the three moulds (Cardinal Parameter Model, CPM, with n=2, (Rosso et al., 1995)). *E.repens* and *P.corylophilum* showed a similar range of cardinal values (T_{min} around 1°C and T_{max} around 27°C), whereas *A.niger* showed the same pattern but at higher temperatures (T_{min}=6.97°C and T_{max}=45.66°C). A different gamma term structure was applied to describe the effect of a_w on proliferation rate: a CPM (n=2) structure for *E.repens*, and a linear function for *A.niger* and *P.corylophilum*.

CONCLUSIONS AND IMPACT OF THE STUDY

The effect of water activity, storage temperature and pH on proliferation rate of three moulds was assessed on Malt Extract Agar media and then a predictive model was developed. This model could be now implemented in a probabilistic framework to determine bakery product shelf-life (Dagnas and Membré 2013).

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Role of colony formation on the growth dynamics of Salmonella Typhimurium and Escherichia coli at suboptimal conditions

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OBJECTIVE(S)

The impact of food structure on microbial growth has recently been acknowledged. Colony morphology also plays an important role in growth dynamics; colonies can either be immersed within the system or grow on the surface. Growth in structured media has been reported slower than that in liquid systems (Theys et al. 2008, Wilson et al. 2002), which suggests that growth in structured systems may not be well predicted by models derived from broth systems (Brocklehurst et al. 1997). The dynamics of *Salmonella* Typhimurium and *Escherichia coli* were studied as a function of temperature, given different growth morphologies: (i) planktonic cells, (ii) immersed colonies, and (iii) surface colonies. Both microorganisms were grown at the suboptimal temperature range [8-22ºC].

METHODS(S)

The dynamics of *Salmonella enterica* serovar Typhimurium SL1344 and *Escherichia coli* K12 MG1655 were studied. Both microorganisms were grown in petri dishes containing, respectively, Tryptic Soy Broth or Brain Heart Infusion. In order to mimic a structured environment, 5% gelatin was added. The petri dishes were incubated, without shaking, at temperatures in the range [8-22ºC]. At regular time intervals, microbial concentration was determined. For the structured media, the content of the petri dish was transferred to a stomacher bag and homogenized after a short liquefaction of the medium at 37ºC. Cell density was determined via viable plate counting on TSB or BHI supplemented with 1.5% agar. Plates were incubated at 37ºC for 24 h.

RESULTS

Growth curves were fitted with the model of Baranyi and Roberts (1994). Clear differences can be observed for the maximum cell density in the stationary phase, with the surface colonies having the highest values. The lag phase seems to be shorter for colonies than for the planktonic cultures. From a first glance, temperature similarly affects the growth rate of planktonic cells and colonies. When increasing the temperature, a similar increasing trend is noticed for the growth rates of the different morphologies. The effect of the temperature on the growth rate was modelled by using the secondary square root model of Ratkowsky et al (1982). As expected, differences considering the growth rate appear to be rather limited, which was affirmed when fitting the Ratkowsky equation. Only small differences can be observed for both parameters b and T_{min}. The observation of similar growth rates for planktonic cultures and colonies deviates from what is reported in literature, where a difference is observed, i.e., $\mu_{planktonic} \geq \mu_{immersed\ colonies} \geq \mu_{surface\ colonies}$. Most likely, this difference is due to shaking in other studies, which results in a nutrient and oxygen rich environment, in contrast to the diffusion-limited gel system. It also has to be pointed out that either in this study or the mentioned literature, the evolution of the global population was monitored, and not the growth dynamics of the colony itself, which is a key factor to be addressed.

CONCLUSIONS AND IMPACT OF THE STUDY

This research shows that the morphology (planktonic vs. colony) has a negligible effect on the growth

dynamics of *Salmonella* Typhimurium and *Escherichia coli* as a function of temperature. This study contributes to the state-of-the-art regarding growth dynamics in solid systems and will lead to improved predictive models and food safety assurance systems.

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Monitoring minced pork spoilage using GC/MS and chemometrics

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OBJECTIVES

The lack of general agreement on the early signs of incipient spoilage for meat is well recognized. This issue makes all more difficult the task to evaluate it objectively mainly due to changes in the technology of meat preservation (e.g., vacuum, modified atmosphere, etc.).. Nowadays the evaluation of the degree of meat spoilage is usually made either subjectively, based on sensory assessment or by microbiological analyses. It is therefore crucial to have valid methods to monitor freshness and quality. The use of microbial metabolites acquainted from HPLC or GC/MS, as consequence of microbial growth in meat, has been recently proposed as a potential means for assessing meat quality (Argyri et al. 2011). Indeed the potential use of gas chromatography/mass spectrometry (GC/MS) has been recently put forward for the rapid and quantitative monitoring of meat spoilage. This was achieved by incorporating in data analysis advanced statistical methods (discriminant function analysis, chemometrics, etc) and intelligent methodologies (artificial neural networks, fuzzy logic, etc) may be used for qualitative and quantitative indices in parallel with unsupervised or supervised learning algorithms.

METHODS

Minced pork meat was stored aerobically and under modified atmospheres at five different temperatures (0, 5, 10, 15 and 20°C) and the microbiological analysis (total viable counts, lactic acid bacteria (LAB), *Pseudomonas* spp. and *Brochothrix thermosphacta*) was performed in parallel with GC/MS analysis and sensory evaluation. The volatile compounds of meat were isolated by the headspace (HS) solid phase micro-extraction (SPME) method (HS/SPME). GC/MS analyses were performed on an Agilent 7890A gas chromatograph coupled to an Agilent 5973C mass spectrometer.

The spectral data collected from GC/MS were subjected to Factorial Discriminant Analysis (FDA) in order to predict the quality of a sample that was pre-characterized as Fresh (F), Semifresh (SF) or Spoiled (S) from the sensory panel. Moreover, in an effort to estimate the counts of the different microbial groups, the spectral data were regressed using a partial least squares regression (PLS-R) model onto total viable counts (TVC), *Pseudomonas* spp., *Br. thermosphacta*, and LAB. The leave-one-out cross validation technique was applied to evaluate the performance of the model. The performance of the regression model was quantified by the calculation of the bias (B_f) and accuracy (A_f) factors, the standard error of calibration (SEC), standard error of prediction (SEP).

RESULTS

Results showed that FDA provided good discrimination of minced pork samples regarding spoilage status. The classification for air and map samples (confusion matrix) provided 93.62% and 89.13% correct classification and 89.26% and 76.09% when cross-validated, respectively. Additionally, results demonstrated good correlation of microbial groups with spectral data as it can be seen by the performance criteria. The

values of B_f were generally close to unity, indicating good agreement between observations and estimations, with almost no structural correlation between estimations and observations. In addition, the values of the accuracy factor indicated that the average deviation between estimations and observations of the various microbial groups enumerated ranged from 6.9 % for TVC to 11.4% for LAB for aerobic samples, while for map samples results showed similar trend.

CONCLUSIONS AND IMPACT OF THE STUDY

These results indicate that the HS/SPME-GC/MS analysis can provide useful information about the dynamic changes of the volatile metabolic compounds being present in the meat substrate during storage and provide estimations about the microbial populations and the sensory scores.

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Monitoring minced pork spoilage using HPLC and chemometrics

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OBJECTIVE

Quality is a subjective and sometimes elusive term. Freshness of meat muscles is generally considered as the most important contributor to quality. Today, there is no general agreement on early criteria indicative of meat quality. Thus, despite the more than 50 chemical, physical and microbiological methods, which have been used over decades for the detection and measurement of meat spoilage, no definitive biomarkers have been identified. The relationship between microbial growth and chemical changes occurring during meat storage has been continuously recognized as a potential means to reveal indicator(s) that may be useful for quantifying beef quality or freshness.

METHODS

Minced pork meat was stored aerobically and under modified atmospheres at five different temperatures (0, 5, 10, 15 and 20°C) and the microbiological analysis (total viable counts, lactic acid bacteria (LAB), *Pseudomonas* spp. and *Brochothrix thermosphacta*) was performed in parallel with sensory evaluation and HPLC analysis of the organic acid profiles. The spectral data of approximately 164 meat samples, collected from HPLC were subjected to factorial discriminant analysis (FDA) in order to predict the quality of a sample that was pre-characterized as Fresh (F), Semifresh (SF) or Spoiled (S) from the sensory panel. Moreover, in an effort to estimate the counts of the different microbial groups, the spectral data were regressed using Support Vector Machines onto total viable counts (TVC), *Pseudomonas* spp., *Br. thermosphacta*, and LAB. The performance of the regression models was quantified by the calculation of the bias (B_f) and accuracy (A_f) factors, the standard error of calibration (SEC), and standard error of prediction (SEP).

RESULTS

The analysis of spectral data from HPLC resulted in the selection of 16 discrete peaks. From the metabolomics' point of view, the information contained in all peaks is critical for the characterization of spoilage. In this respect, the whole profile (all the peaks) as derived from spectral data was taken into consideration. The results demonstrated changes in the chromatographic areas under the peaks of the eluted acids, that were associated or not with storage conditions (e.g. temperature, packaging). The FDA provided good discrimination of minced pork samples regarding spoilage status. The classification for air and map samples provided 97.6% and 94.7% correct classification when cross-validated, respectively. Additionally, results from SVM demonstrated good correlation of microbial groups with spectral data as it can be seen by the performance criteria. The values of B_f were generally close to unity, indicating good agreement between observations and estimations, with almost no structural correlation between estimations and observations. In addition, the values of the accuracy factor indicated that the average deviation between estimations and observations of the various microbial groups ranged from 11.9 % for TVC to 15.1% for LAB for aerobic samples, while for map samples results showed similar trend.

CONCLUSIONS AND IMPACT OF THE STUDY

The obtained results indicate that the HPLC analysis of the organic acids can be utilised as a potential

technique for (i) the qualitative classification, and (ii) prediction of the microbial load of minced pork meat regardless of storage conditions.

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Prediction of Listeria monocytogenes Counts Using an Electronic Nose

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OBJECTIVE(S)

The objective of the study was to establish a rapid, non-destructive and odor fingerprinting-based method for detection and prediction of *L. monocytogenes* using an electronic nose (EN) and hence to develop an non-invasive method for data acquisition for predictive microbiology.

METHOD S(S)

In this study, *L.monocytogenes* was selected as the main object and the volatile metabolites of *L. monocytogenes*which released in different media, in different incubation time and the impact of the presence of other bacteria *(Slentus)* were detected by odor fingerprint technology based on EN. Chemical metrology and statistical methods were used for statistical analysis. To explore the potential applications of the odor fingerprinting technique for quantitative prediction microorganisms, the following approaches were conducted: (1) the prediction model was constructed between EN signal responses and *L.monocytogenes* numbers combined with partial least squares (PLS); (2) one multiple linear regression prediction model was established between the logarithm of *L.monocytogenes* concentration and the relative content of five kinds of volatile metabolites detected by EN; (3) Two PLS models which were established by *L. monocytogenes* and *Sdentus* under pure culture conditions were used to predict the concentration of two bacteria in mixed culture system (Mixed *L.monocytogenesmih Slentus).*

RESULTS

The results showed that the linear relationship of *L. monocytogenes* concentration and electronic nose signals was established with linear range from 10^6 CFU/ml to 10^s CFU/ml, and that the correlation coefficient (R^2) was 0.9937. The relative content of five kinds of volatile metabolites (2,3-Butanedion-e, *xi*\ 3-Methyl-butanone, *xy,* 3-Hydroxy-2-butanone, *xy,* 2,5-Dimethyl-pyrazine, *x/,* Benzaldehyde, *xj)* were taken as variables, and the multiple linear regression model of bacterial concentration ($y = -1.11562 + 3.73019\ x_x + 11.84649\ x_2 + 13.01102\ x_3 + 9.05566\ x_4 + 13.46266\ x_5$) was successfully established with significant *F-test (p<0.05).* Moreover, the PLS model of *L.monocytogenes* can be used to predict the concentration of *L. monocytogenes*'in the mixed system and the prediction result was good.

CONCLUSIONS AND IMPACT OF THE STUDY

The EN system could be used as a simple and non-invasive methods for data acquisition for predictive microbiology, and would have a prospective application in the filed of food safety risk assessment in the future.

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A comparison of artificial neural networks and traditional mathematical method for modeling the spoilage bacterial growth in MAP packaged chilled beef

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OBJECTIVE(S)

Organisms involved in the beef spoilage usually are *Pseudomonas, Lactobacillus, B. thermosphacta* and Coliform (Borch & Kant-Muermans, 1996). The modified atmosphere packaging (MAP) and chilled conditions (0-4°C) are proved to be fairly efficient to extend its shelf-life (Pennacchia et al., 2011). Therefore, predicting the spoilage bacterial growth in the MAP packaged chilled beef is a meaningful work that is closely related to daily life. In present study, an efficient Artificial Neural Networks (ANN) method was developed to predict the microbial growth in beef. The targeted environmental factors were: temperature (-2, 0, 5 and 10°C) and Modified Atmosphere Packaging (MAP) air component (65% 0_2, 35%CC>2 and 80% O2, 20%CC>2). To prove its effectiveness, the traditional mathematical models which combined Modified Gompertz (MG) equation and Response Surface Methodology (RSM) were applied to the same datasets.

METHOD S(S)

For traditional modeling: the growth database of bacteria under each condition was modeled by the MG model (Equation 1).

$$log(N_t) = log(N_0) + log\left(\frac{N_{max}}{N_0}\right) \times exp\left(-exp\left(\frac{e*\mu_{max}}{log\left(\frac{N_{max}}{N_0}\right)} \times (\lambda - t) + 1\right)\right) \quad (1)$$

λ is the lag phase extension(days); μ_{max} is the maximum exponential microbial growth rate(days^{-1}); t is the storage time(days); $log(N_0)$ is the initial bacterial density(log cfu/g); $log(Nt)$ is the counts of bacteria in t days and $log(N_{max}/N_0)$ is the differential between the maximum counts and the minimum counts of microbes(log cfu/g) .Then the combined effect of two variables (temperature and MAP) was correlated with growth parameters($log(N_0)$, $log(Nmax/N_0)$, μmax and λ) got from the primary model, the RSM is in the following form(Equation 2):

$$Y = b(1) + b(2) \times M + b(3) \times T + b(4) \times M \times T + b(5) \times M^2 + b(6) \times T^2 \quad (2)$$

b(l),b(2J... b(6) are the model coefficient parameters which are estimated by multiple linear regression analysis using the least squares method; *M'vs*, the percentage composition of $C0_2$; *T'^*, the storing temperature.

ANN modeling: The ANN model applied in the study was a three-layer BP network (Basheer Hajmeer, 2000). Two types of ANN model were constructed which were three-vector model and four-vector model. The input layer in the three-vector model was consisted of three neurons: storing time, temperature and air components in the MAP. The output layer had one neuron, representing the counts at its corresponding time points. Compared to three-vector model, the four-vector ANN model had an extra neuron in the input layer representing the bacterial species, by which was able to predict all the bacterial growth in the beef When the counts under each storing condition were obtained, the MG equation was applied to describe the growing curve.

RESULTS

Both the ANN models and the mathematical method were closely matching to the modeling datasets ($R^{2=}$0.988 for three-vector ANN model, 0.953 for four-vector model and 0.979 for mathematical model). Compared to mathematical model, the ANN models showed significantly lower error (RMSE:0.4548 for three-vector model, 0.3572 for four-vector model and 0.6664 for mathematical model) and lower bias(7*/:1.0210 for three-vector model, 1.0493 for four-vector model and 1.1584 for mathematical model) in the testing datasets. And the more variables did not affect the accuracy of four-vector model significantly in testing^ 1.1878 for three-vector model and 1.1886 for four-vector model) datasets.

CONCLUSIONS AND IMPACT OF THE STUDY

In both modeling and testing datasets, the two ANN models were demonstrated to got the better prediction. And the four-vector ANN model which adding an extra neuron into the input layer was proved to be able to present all the five bacteria(including TVC) growth in a single model. The precision of ANN model did not decline when the vector changed from three to four. Since there were four major spoilage bacteria in chilled beef, the four-vector model constructed was able to show a full-scale prediction by which all bacterial counts and growth can be simulated. Therefore, the ANN method can be an alternative approach to cope with more environmental factors as well as the interaction between microbial flora in beef stuff.

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A study of the Gamma hypothesis: Predicting combined effects of pH and salt on growth of Listeria monocytogenes

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OBJECTIVE(S)

Controlling factors to limit microbial growth are frequently applied in foods to achieve an overall level of protection against foodborne pathogens and spoilage flora. To assess the combined effect of the controlling factors on microbial growth, some predictive models assume independent additive effect of the controlling factors [1] (Gamma hypothesis: absence of synergistic effects), whereas other models apply the socalled LeMarc interaction term [2] to quantify possible synergistic effects between the controlling factors near the growth boundary. The purpose of this study was to investigate if synergistic effects on growth of *L. monocytogenes* exist between the controlling factors pH and salt, and to investigate if the LeMarc interaction term is a suitable method to quantify this effect in a model, and if the term possible could be improved.

METHODS(S)

The growth mediums used for the growth experiments were BHI broth prepared with varying NaCl concentrations (0-9 w/w%), and varying pH (4-8) according to a complete factorial experimental design. Growth of *L. monocytogenes* strain ATCC19115 was followed by OD600 measurements in an automatic reader Bioscreen C system (Lab systems Corp. Helsinki, Finland). The maximum specific growth rates for each combination of salt and pH were derived from the OD growth curves. From the experimental data independent secondary models for influence of pH and salt on growth of *L. monocytogenes* were derived and the performance of each independent secondary model was compared. The best performing independent secondary models were included in combined non-synergy and synergy models and their performance were compared when tested on the experimental data. One of the synergy models includes a novel interaction term. The best performing combined model was finally validated on a separate data set.

RESULTS

The results show that the combined model including the LeMarc interaction term performed better than the non-synergy model, and the model with the novel interaction term performed even better for *L. monocytogenes*, when a threshold value in the novel interaction term was adjusted so that the synergistic effect commence at a lower level of combined environmental stress than prescribed in the LeMarc interaction term.

CONCLUSIONS AND IMPACT OF THE STUDY

Our results confirm that there are synergistic effects of pH and NaCl near the growth boundary for *L. monocytogenes* and that the LeMarc interaction term is a suitable way to describe the combined inhibiting effect, even though the model using the novel interaction term performed better for our data. Our interpretation is that the arbitrary threshold value of combined environmental stress of 0.5 in the LeMarc interaction term controlling when synergy appear between controlling happens, is not a general number, but

happens to be suitable for *L. monocytogenes*, even though our results indicate that a value of 0.39 is better. Other studies have tried to include the LeMarc interaction term in models for other bacteria species, but did obtain better results, when adding the LeMarc interaction term to their model of *Bacillus cereus* [3]. A future hypothesis to investigate could be that all bacterial species are actually affected by synergistic effects of controlling factors, but they are maybe not equally sensitive to level of combined environmental stress at which synergy commence as conditioned in the LeMarc interaction term. Future research could quantify this possible difference in sensitivity between different bacteria using the novel modified interaction term.

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Modeling the Listeria innocua lag phase and its variability

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OBJECTIVE

Most classical microbial predictive models predict static values, which have proven effective over decades in "overkill" treatments designed to safeguard the microbiological safety of foods; they predict only the mean number of survivors and do not take into account the datum dispersion. Another significant fault of classical models of microbial growth is that while they predict the growth rate with sufficient precision, they fail to predict the lag phase duration, which is essential for estimating the food shelf-life. Thus these static models must be improved in order to provide realistic "best if used by" dates on packaging, which if too conservative lead to economic losses but if too liberal lead to uncertainty about microbiological risk.

As a step towards generating improved models to predict microbial growth, this research aimed to apply mathematical tools to the study of the variability of microbial inactivation and of the lag phase of surviving microorganisms. This study sought to improve our understanding of the efficiency of food preservation techniques and to generate data that may help improve the precision of predictive models of microbial growth.

METHODS(S)

Firstly, the optimized working (determination of kinetic parameters) conditions were used to study the variability in the number of survivors of *Listeria innocua* after heat inactivation treatments of different intensity, which were designed to achieve different degrees of decimal reduction from 0 to 7 cycles. To ensure that the full extent of variability could be measured, each experiment was carried out using at least 75 samples.

The second part of the project examined the variability in the lag phase duration of cells that survived the heat treatments described above. Cultures of survivors were diluted down to single cells, which were then incubated at refrigeration and thermal abuse in order to estimate the lag phase.

A final part of the project was to model the lag time of *L. innocua*, and its variability (described as standard deviation) as a function of heat shocks, number of survivors, and growth temperature.

RESULTS

Microbial inactivation shows a quantifiable variability. The more intense the preservation treatment, the smaller is the number of surviving organisms, but the greater is the variability in this number. Likewise, the more intense the preservation technique, the longer and more variable the lag phase is. Numerous factors affect lag phase duration and variability, including inoculum size, temperature, and any other factor that might affect the physiological state of the microorganism.

Models predicting the inactivation and lag phase, considering the variability, of *Listeria innocua* have been developed and their validations indicated that all of them may be applied to predict the microbial behavior in a food product with a reasonable accuracy.

CONCLUSIONS AND IMPACT OF THE STUDY

Variability inherent in a preservation treatment should be taken into account, so that the treatment can reduce microbiological risk to the desired level, rather than to the level dictated by chance. This insight should inform any analysis of risks and critical control points, and it should be applied to any quantitative assessment of microbial risk. To ignore the variability in microbial inactivation is a dangerous oversight.
Under certain conditions, lag phase duration and variability can become the primary determinants of food shelf-life. This happens when the inoculum is quite small, on the order of units, tens or few hundreds of viable cells, and when growth conditions are far from optimal. Since both situations are typical of many foods, the work in this study may prove particularly useful for predictive microbiology in the food industry.

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A Theoretical Mathematical Model for the analysis of lettuce quality in a Food Production Chain using Fuzzy Cognitive Maps.

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OBJECTIVE(S)

The objective of this study was the development and the use of Fuzzy Cognitive Maps (FCM) in modeling a Decision Support System which diagnoses the importance of Critical Control Points for the safety during the food of a vegetable (lettuce). Minimally processed vegetables, such as lettuce, have recently undergone an increase in consumer demand because of their healthy image and convenience of use. Some of these products can be contaminated by potential pathogens, as some have been implicated in an increasing number of outbreaks of foodborne illnesses (Kokkinos et al., 2012). Bacterial pathogens such as *Salmonella enterica*, *Escherichia coli* O157:H7, *Bacillus cereus*, *Listeria monocytogenes*, are especially of major concern due to the environmental occurrence of these bacteria, in different stages during the food production chain (Oliveira et al., 2011). In this study a simple but illustrative way of how FCMs can be used in predicting problems arising in lettuce production chain, will be presented.

METHODS

The methodology described extracts the knowledge from experts and exploits their experience of the process. (Groumpos et al., 2012). Each expert based on his/her experience knows the main factors that contribute to the decision. Experts describe the existing relationship firstly as “negative” or “positive” and secondly, as a degree of influence using a linguistic variable, such as “low”, “medium”, “high” etc. Its term set T (influence) is suggested to be comprised of nine variables. Using nine linguistic variables, an expert can describe the influence of one concept on another in detail and can discern it between different degrees. The nine variables used here are: T(influence) = {negatively very strong, negatively strong, negatively medium, negatively weak, zero, positively weak, positively medium, positively strong, positively very strong}. With this method the purpose is to diagnose and predict the effect of different factors during the lettuce production chain in their contribution to a final safe lettuce. The factor concepts are nine and describe the critical control points during a lettuce production chain. Specifically the concepts selected were the following: C1: Labor, Manpower, C2: Quality and Safety of Food System, C3: Location – Surroundings of the growing field, C4: Lettuce Nursery, C5: Produce Land, C6: Harvesting the crop, C7: Postharvest Processing, C8: Transportation, C9: Point of Sale, C10: Safe lettuce

RESULTS

Each individual Food Science expert expresses the cause and effect relationship that exists between the nodes of FCM, concerning the lettuce safety. Then the degree of correlation between two nodes is determined (the exact weight value Wij). The defuzzyfication method ‘Centre of Area’ (COA) is used in order to convert the experts’ estimations into a numerical weight Wji, belonging to the interval [-1,1]. If C1 is medium and C2 is weak and C3 is medium and C4 is medium and C5 is strong and C6 is strong and C7 is strong and C8 is weak and C9 is zero then OUT is medium. After only few iteration steps the FCM reaches an equilibrium point. So the calculated value of the decision concept corresponds to the 43% of the output.

CONCLUSIONS AND IMPACT OF THE STUDY

The interesting results obtained here show that software tools like Food Science Support Systems using theories of FCMs, which have not been yet used in Food Science, can be explored and problems that can be arise during the food production chain can be studied further in order to indicate the importance of some critical control points during the food production.

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Simulation of Listeria monocytogenes single cell colonial growth

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INTRODUCTION

The production hygiene encountered in the dairy industry is typically high and contamination of products with human pathogenic or spoilage microorganisms is sporadic. Furthermore, when contamination occurs, the concentration of these microorganisms in the products is typically very low. When dealing with low initial bacterial concentrations, a high variability in population lag time should be expected and application of stresses (e.g. pH and organic acids) further increases this variability. Deterministic models can describe the effect of stresses on microbial responses but they are not optimal for responses of very small initial concentrations of microorganisms, since cell and strain variability is not taken into account (Koutsoumanis, 2008; Poschet et al. 2003). In the case of cottage cheese, the pH encountered in the product is close to the growth boundary of *Listeria monocytogenes* and variability in single cell lag time is expected. The aim of this study was to model growth of one individual cell until a colony size of 100 cells. Cell variability was taken into consideration by application of a stochastic modelling approach. Detailed data of the first few division times of individual cells of *L. monocytogenes*, under conditions similar to those found in cottage cheese, was used to develop the model. A similar approach has previously been successfully applied by Pin and Baranyi (2006) by use of data from single cell growth in a flow-chamber.

METHODS

Growth of individual cells of *L. monocytogenes* was studied by phase contrast time-lapse microscopy according to the method of Koutsoumanis and Lianou (2013). The agar-based growth environment was adjusted to mimic cottage cheese (1.0% NaCl, pH 5.30, 800 ppm lactic acid) and cells were monitored for approximately 18 h at 25°C. Images were taken every 10 minutes. From the images, information of the first to the fourth division time of each cell was extracted using a tree structure to track and name the cells. Subsequently, data were used to parameterise gamma distributions to be applied in the simulation model. The principle of event based modelling was applied to build the simulation growth model for individual cells.

RESULTS

From the images taken, a total of 165 observations (division times) were recorded and used to parameterise individual gamma distributions for each division time of individual cells. The observed distributions of division times were significantly different for the first four generations. Thereafter we could not show a difference, which is also in accordance with the observations of Koutsoumanis and Lianou (2013). This was reflected in the model by having separate distributions for the first four divisions and a fifth distribution for the remaining generations. Simulated growth curves were evaluated against the observed growth data with time to at which 20 cells used as benchmark. A comparison of 250 simulations with ~70 observations showed satisfying agreement between observed and simulated data at the benchmark value.

CONCLUSIONS AND IMPACT OF THE STUDY

The developed simulation model performed well with a high agreement between simulated growth curves and observed growth. The model was also able to simulate growth by the use of tail values, and both fast growing and slow growing cells were represented by the simulation-model. When dealing with risk assessment the fast growing cells are of main interest since they have the highest potential of posing a risk. It is therefore important that the model can simulate these cells without over- or under representing their occurrence.

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Current statistical models employed to describe the distribution of Cronobacter spp. within lots of PIF and evaluation of the effectiveness of the sampling plan implemented

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OBJECTIVE

The statistical distribution of pathogens in lots of food material is unlikely to be random as in many cases bacteria contaminate lots in clusters or group of individual cells (Jarvis, 2008). As to describe the degree of over-dispersion of pathogens in food, ICMSF (2009) developed a mathematical model based on the assumption that bacteria follow a Poisson lognormal (PLN) distribution within-lots. Recent studies (Mussida *et al.*, 2013b) introduced the Poisson-logarithmic (Plog) distribution as to describe microbial clustering. The Plog model makes the assumption that microorganisms contaminate food material in clusters randomly distributed in a Poisson process, while the number of individual cells within the clusters is distributed independently in a logarithmic distribution. The Plog distribution is mathematically equal to a negative binomial (NB) distribution (Anscombe, 1950; Mussida *et al.*, 2013b). The aim of this study was to evaluate the effectiveness of the sampling plan implemented by European regulators (30 samples, 10-g) for *Cronobacter* spp. in powder infant formula (PIF) based on the assumptions that this pathogen follows a PLN or a Plog distribution within lots of PIF. Finally, operating characteristic (OC) curves were generated and compared.

METHODS

In order to describe over-dispersion of *Cronobacter* spp. in PIF, we assumed that the variance-to-mean ratio (I) is fixed at any mean level of contamination μ and sample of mass w employed. Based on the PLN distribution, in order to ensure our basic assumption of a fixed I, the mathematical procedure introduced by Mussida *et al.* (2013a) was adopted while for the Plog distribution the methodology introduced by Mussida *et al.* (2013b) was employed. Finally OC Curves were generated and compared assuming increasing values of I as to describe the increasing effect of clumping of *Cronobacter* spp. in PIF. Further, OC Curves were generated based on the assumption that the within-lot variability (S) of the PLN distribution (ICMSF, 2009) and the reciprocal of the exponent k of the NB distribution are fixed at any μ and w employed.

RESULTS

As the value of I increases, the OC curves generated based on both the PLN and Plog distribution, depart from the one based on a Poisson distribution and both the consumer's (CQL) and producer's quality levels (PQL) increase. Having fixed I, the OC Curves based on a Plog distribution result more conservative (i.e., OC curves shifted to the right) when compared to the OC curves based on a PLN distribution. Both the OC Curves based on the assumption of fixed values of S and $1/k$ at any μ and w employed for the PLN and Plog distribution respectively, converge to a Poisson distribution at the PQL, while the CQL increases as the values assumed for S and $1/k$ increase.

CONCLUSIONS AND IMPACT OF THE STUDY

This study allowed us to evaluate the performance of the sampling plan implemented for *Cronobacter* spp. by comparing OC curves based on a PLN and Plog distributions and indices of dispersion commonly used

for describing effect of clustering of pathogens in food. The results demonstrate that the current sampling plan implemented for *Cronobacter* spp. in PIF is not efficient if a high degree of clustering occurs, as both the CQL and PQL result unacceptably high. For more sound conclusions, quantitative data based on an enumeration method should be available as to determine the degree of over-dispersion of this pathogen in PIF.

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Optimal experiment design for discrimination in predictive microbiology models

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OBJECTIVE(S)

In the field of predictive microbiology, mathematical models play an important role for describing microbial growth, survival and inactivation. Often different models are available for describing the microbial dynamics in a similar way. However, the model that describes the system in the *best* way is desired. In this respect, *optimal experiment design for model discrimination* (OED-MD) is an efficient tool for discriminating among rival models.

METHODS

The idea is to use optimal experiment design to discriminate between available models. To this end, new experimental conditions are designed that maximize the difference between the predictions of the different models. The difference in the model predictions enables the selection of the model that fits more accurately the experimental data. There exist in literature several criteria which take this difference into account. In the current work, two different criteria are used, i.e., (i) the T_{12}-criterion employed by Ucinski and Bogacka (2005) and (ii) the Schwaab-approach proposed by Schwaab et al. (2008). The first criterion performs discrimination in a simultaneous way whereas the second does it in a sequential manner. In contrast to a typical set of experiments at constant levels, the dynamic experiments designed in the current work enable the discrimination between two models in a cost and labor efficient way.

RESULTS

For describing the influence of temperature on the microbial growth rate there exist several models in predictive microbiology. Two of these models are the CTMI (Cardinal Temperature Model with Inflection (Rosso et al., 1993) and the aCTMI (adapted CTMI (Le Marc et al., 2002)). While the CTMI model assumes a one-phase linear relation between the square root of the growth rate and the temperature in the suboptimal temperature range, the aCTMI starts from the assumption of two phases in this temperature region. Up till now, it has been assumed that the CTMI is generally valid for *E. coli* K12. In this work, the possibility of discriminating between the CTMI and aCTMI models has been evaluated in silico.

First, preliminary experiments have been performed to yield parameter estimates which are good enough to start the model discrimination procedure. Afterwards both criteria have been exploited and found to provide dynamic temperature profiles capable of discriminating between the CTMI and aCTMI model. Although both approaches perform almost identically, the Schwaab-procedure yields less biased parameter estimates and requires less computational effort. Obviously, the T-procedure was not designed for obtaining good estimates whereas for the Schwaab-approach this appears to be the case.

CONCLUSIONS AND IMPACT OF THE STUDY

Based on the in silico study, the proposed experimental design methods for model discrimination can be used to evaluate if the diverging kinetics (observed for *Listeria* strains), also hold for *E. coli* K12. Hence, the above-mentioned discrimination methods provide an efficient way to select an appropriate predictive

microbiology model with less experimental effort.

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Estimation of the within and between-lot mean level of contamination and variability of Cronobacter spp. in PIF from prevalence data

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OBJECTIVE

The current statistical model employed to described the between and within-lot distribution of *Cronobacter* spp. in powder infant formula (PIF) is based on the assumption that the pathogen follows a Poisson lognormal (PLN) distribution within lots of PIF and that the mean level of contamination between lots follows a lognormal distribution (FAO/WHO, 2007). Mussida *et al,* (2013a) mathematically assed the implications and limitations of the assumptions introduced by FAO/WHO (2007), such as employing a fixed within-lot variability (5) at any mean level of contamination M and sample of mass w employed. As the quantitative data obtained by the manufacturer in accordance with the sampling plan implemented to analyze lots of PIF is based on an absence/presence test, both the within (M) and between-lot (M_b) mean level of contamination, as both the within (5) and between-lot variability (S_b) are often unknown. Therefore, the aim of this study was to develop a new statistical methodology in order to estimate the within-lot mean level of contamination M from prevalence data and finally estimate the between-lot mean level of contamination expressed both as counts (ju_b) and in the log10 scale (M_b) and respective between-lot variability $\boldsymbol{a}_b$ and S_b.

METHODS

Based on a set of theoretical prevalence data, which was generated as an example of industrial data, we determined the within-lot $\boldsymbol{p}$ assuming that the distribution of *Cronobacter* spp. within-lots of PIF follows a Poisson-logarithmic distribution and that the variance-to-mean ratio / is fixed (Mussida *et al.*, 2013b). Since for most of the lots it was not possible to determine $\boldsymbol{p}$ as no positive samples were detected, in order to estimate $\boldsymbol{p}_b$ and S_b we employed a lognormal distribution capable of fitting left-censored data (Zhao and Frey, 2004). Thus, we assumed that *Cronobacter* spp. contaminate all lots of PIF, and where no positive samples were detected, the mean level of contamination of the pathogen was below the limit of detection. As pooling of the sample is common practise in the industry and therefore the number of positive samples detected in each lot is no longer available, we assessed several sizes of a lot, where lot was defined as the amount of powder produced in a certain period of time.

RESULTS

Assuming as lot the amount of powder produced in 6-hours or 1 day, it was not possible to estimate $\boldsymbol{p}$ as the number of samples tested was too small. Assuming as size of a lot the amount of PIF produced in 1 week, 60% of the data were left-censored and employing values of/ = {5 and 100} we estimated $\boldsymbol{P}_b$ = {0.00019 and 0.0016} (cells/g), M_b = {-3.914 and -2.977}, $\boldsymbol{a}_b$ = {0.0002 and 0.00175} and S_b = 0.389. Thus the estimate of S_b is not influenced by the value of/ employed as for p_b, M_b and a_b. The same statistical analysis and similar results were obtained assuming a greater size of a lot (i.e., month).

CONCLUSIONS AND IMPACT OF THE STUDY

The statistical procedure introduced in this study allowed us to estimate the mean level of contamination and variability within and between-lots of PIF. Yet, as the degree of over-dispersion of *Cronobacter* spp. is unknown, increasing values of I were employed as to describe the increasing clustering of the pathogen within lots of PIF. The employed lognormal distribution that takes into account left-censored data, allowed

us to estimate the between-lot mean level of contamination and variability.

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