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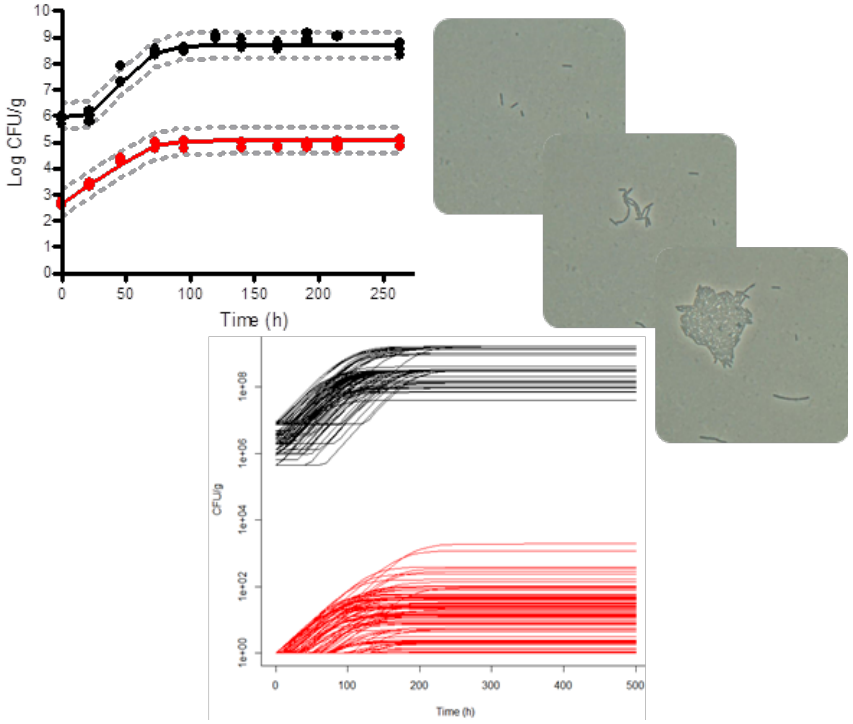
Summary of PhD Thesis

Predictive Food Microbiology

– new tools for risk assessment and dairy product development



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Summary

Predictive Food Microbiology – new tools for risk assessment and dairy product development

Listeria monocytogenes is a well-known food borne pathogen that potentially causes listeriosis. No outbreaks or cases of listeriosis have been associated with cottage cheese, but several confirmed cases and outbreaks in the EU and the US have been related to dairy products made from raw or pasteurised milk. This, in combination with the fact that cottage cheese support growth of *Listeria monocytogenes,* induces a documentation requirement on the food producer. In the EU regulatory framework, mathematical models are recognised as a suitable supplement to traditional microbiological methods. The models can be used for documentation of compliance with microbiological criteria for *Listeria monocytogenes* under reasonably foreseeable conditions.

Cottage cheese is a fresh, fermented dairy product. It consists of a fermented cheese curd mixed with a fresh or cultured cream dressing. The product contains considerable concentrations of lactic acid bacteria from the added starter or aroma cultures. The presence of these microorganisms induces some complexity to the product, since the lactic acid bacteria metabolites and e.g. bacteriocins exhibit an inhibitory effect towards co-culture microorganisms such as *Listeria monocytogenes.* During storage at temperatures allowing the mesophilic lactic acid bacteria to grow (> 8-10°C), a pronounced inter-bacterial interaction and growth inhibition of co-culture *Listeria monocytogenes* was observed. These observations emphasised the need for inter-bacterial interaction models when predicting the growth response of *Listeria monocytogenes* in fermented dairy products.

The objective of the PhD-project was to develop new, or extend existing mathematical models to be used for risk assessment and product development. When the project was initiated, none of the existing predictive models were found to appropriately describe the simultaneous growth of lactic acid bacteria from the added starter or aroma culture and *Listeria monocytogenes* in cottage cheese.

New, deterministic growth models were developed for *Listeria monocytogenes*, starter lactic acid bacteria and aroma lactic acid bacteria. The new cardinal parameter type growth models included the effect of temperature, pH, NaCl, lactic and sorbic acid. The models were developed based on growth data obtained from absorbance measurements in liquid laboratory media and growth data obtained in cottage cheese with fresh or cultured cream dressing. An important step in the modelling procedure was the calibration of the reference growth rate (μ_{ref}, h^{-1} at 25°C) which was strongly affected by the dominating lactic acid bacteria culture. By combining the developed secondary growth models with the empirical Jameson approach, good predictions of the simultaneous growth of *Listeria monocytogenes* and lactic acid bacteria were obtained. Both growth rate and maximum population densities of *Listeria monocytogenes* was accurately described under constant and dynamic storage temperatures (between 5°C and 15°C).

The inter-bacterial interaction was clearly important to include when predicting growth response of *Listeria monocytogenes* in fermented dairy products. Alternative, semi-mechanistic,

modelling approaches were evaluated based on methods applied in the fermentation technology. The dynamics of lactic acid concentration and product pH was related to growth of lactic acid bacteria by the yield factor concept. The ability to predict the maximum population density of *Listeria monocytogenes* in cottage cheese based on dynamic lactic acid and pH was evaluated. For cottage cheese with fresh cream dressing, the semi-mechanistic interaction model successfully predicted the maximum population density. Lactic acid and pH was, however, insufficient to describe the growth inhibition of *Listeria monocytogenes* observed in cottage cheese with cultured cream dressing. Improved, mechanistic, prediction of *Listeria monocytogenes* in cottage cheese with cultured cream dressing would require that additional mechanisms were included in the model, such as other metabolites or bacteriocins. Finally, the semi-mechanistic and the empirical Jameson approach to inter-bacterial interaction modelling were compared. The empirical Jameson model consistently performed equally well or better than the more complex semi-mechanistic model.

In order to evaluate the growth response of more realistic concentrations of *Listeria monocytogenes* and to take variability into account, a stochastic approach was applied. The deterministic growth models were used in combination with stochastic input values for bacterial concentration; lag time duration and product characteristics. Good agreement between predicted and observed growth was obtained, when applying broth based lag time distributions for *Listeria monocytogenes* single cells in combination with the relative lag time concept. Furthermore, application of relative lag time distributions from *Listeria monocytogenes* population data provided good predictions of the growth response of only a few *Listeria monocytogenes* cells in cottage cheese at chilled temperatures.

From the results of the present PhD-project it was found that once solid, deterministic, secondary growth models have been developed and validated, they can be modified and/or extended to a range of other modelling procedures. Furthermore, inclusion of inter-bacterial interaction was considered to be an inevitable part when modelling and predicting growth of *L. monocytogenes* in fermented dairy products. In general, simple approaches to describe interaction and growth inhibition (empirical approach), lag time prediction of individual cells (variability in population *RLT*-values) and representation of e.g. variable product characteristics (bootstrapping from empirical distributions) were advocated. It is believed that it is necessary to define some applicable methodologies for the development of growth models for complex products such as fermented dairy products. Model development is a comprehensive process with an almost infinite data requirement and the findings of the present PhD-project is thought to be important in relation to the development of predictive models that are valuable for, and readily applicable in the food industry.

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