SLUTRAPPORT/FINAL REPORT
NR. 2019-158

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Prædiktionsværktøj til risikovurdering og dokumentation af fødevaresikkerhed

Predictive food microbiology tool for risk assessment and documentation of food safety

Mejeribrugets
ForskningsFond

Juli 2019

Final report

for collaborative projects funded via the Danish Dairy Research Foundation (DDRF)

1. Title of the project

UK: Predictive food microbiology tool for risk assessment and documentation of food safety

DK: Prædiktionsværktøj til risikovurdering og dokumentation af fødevaresikkerhed

2. Project manager

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4. Sources of funding

Danish Dairy Research Foundation (Mælkeafgiftsfonden/Milk Levy Fund), Danish Veterinary and Food Administration (Fødevarestyrelsen) and National Food Institute (DTU Food).

5. Project period

Project period with DDRF funding: January 2016 – December 2018.

Total project period, if sub-project within a larger project: The DDRF-funded project is part of a larger project running from August 2015 to April 2019.

6. Project summary

In Danish:

Projektet har fokus på udvikling af matematiske modeller til at forudsige vækst af fordærvelses- og patogene bakterier i en bred vifte af mejeriprodukter. De undersøgte produkter omfattede mælk samt smøre-, skimmel-, modnet-, frisk-, smelte-, fløde-, kemisk syrnet ost og ost i saltlage. Projektet var struktureret i fem underaktiviteter (Opgaver). Opgave 1 nedenfor tilhører den samlede projektperiode, mens opgave 2-5 hører til projektperioden med MFF-finansiering.

Opgave 1: Identifikation af en potentiel hygiejneindikator-mikroorganisme for *L. monocytogenes* i mejerimiljøet og ost baseret på analyse af tilgængelige data og videnskabelig litteratur.

Resultaterne viste, at på trods af den omfattende litteratur, der blev studeret, blev ingen grupper/arter af mikroorganismer, der adskiller sig fra *Listeria*, identificeret som egnede indikatororganismer for at afspejle niveauet af produktionshygiejne for ost eller som indeksorganismer for at vurdere forekomsten af *L. monocytogenes* i oste. (reference nr. 2 i sektion 13).

Opgave 2: Validering og kalibrering af tilgængelige *L. monocytogenes* vækstmodeller til forskellige mejeriprodukter.
3 stk. belastningsundersøgelser er gennemført med *L. monocytogenes* i hvidskimmelost, hvor mælk er podet med bakterien inden fremstilling af ost i laboratoriet. Udover kvantificering af vækst for *L. monocytogenes* og mælkesyrebakterier blev produkterne karakteriseret mht. ændringer af temperatur, pH, mælkesyre og NaCl/vandaktivitet gennem fremstilling og lagring i 30 dage. Forsøgene viste en pH-stigning fra ~4.8 (dag 1) til ~7 (dag 30) og betydelig vækst af *L. monocytogenes* (> 4 log cfu/g) for overflade/rand af ost med *Penicillium camemberti* og *Geotrichum candidum*. Markant mindre pH-stigning og mindre vækst af *L. monocytogenes* blev observeret for ost med *Geotrichum candidum.* For hvidskimmelost blev vækst og overlevelse af *L. monocytogenes* forudsagt med god præcision vha. den tidligere udviklede vækst og vækstgrænse-model fra Østergaard et al. (2014). Det var centralt at anvende målt saltkoncentration samt målte ændringer af temperatur, pH og koncentration af mælkesyre ved forudsigelse af *L. monocytogenes*-vækst i hvidskimmelost (reference nr. 6 i sektion 13).

Yderligere blev ti forskellige, eksisterende *L. monocytogenes*-vækstmodeller testet for deres evne til at forudsige vækst og overlevelse af bakterien i fem forskellige typer af oste. Denne evaluering af modeller blev baseret på 319 forsøg/kurver for vækst og overlevelse af *L. monocytogenes*. Eksisterende, egnede modeller blev identificeret for hvidskimmelostost (DL-kultur; n = 38) og ost i saltlage (O-kultur; n = 18). For blåskimmelost (veined; n = 37) og modnet ost (ripened; n = 11) blev eksisterende modeller kalibreret, hvorefter acceptable forudsigelser blev opnået. For ferske ost (n = 109) kunne en enkelt egnet model ikke identificeres. De opnåede resultater er inkluderet i en videnskabelig publikation (reference nr. 6i sektion 13).

En vækstgrænsemodel for kuldetolerante *pseudomonas* i mælk og hytteost blev udviklet og valideret. Modellen indeholdt effekten af temperatur, pH, NaCl/vandaktivitet, mælkesyre, sorbinsyre samt effekten af interaktion mellem alle disse faktorer.

Opgave 3: Udvidede modeller til at indeholde den vækst-hæmmende effekt af mejeri-specifikke ingredienser.
Der er udarbejdet to videnskabelige publikationer (reference nr. 3 og 4 i sektion 13). Den første publikation beskriver en omfattende model der gør det muligt at forudsige vækst og vækstgrænse for *L. monocytogenes* i kemisk syrnede oste samt i flødeost med pH ned til 4,6. Den anden publikation beskriver en udvidet model, der indeholder den vækst-hæmmende effekt af smeltesalte på vækstpotentialet af *L. monocytogenes* i smelteoste. Begge modeller er fleksible redskaber, der f.eks. kan støtte udvikling af nye produktvarianter, hvor *L. monocytogenes* ikke kan vokse.

Opgave 4: Måle og modellere den anti-listerielle effekt af bakteriociner i fermenterede mejeriprodukter.
Arbejdet med bakteriociner har fokuseret på nisin i forarbejdede oste. En ny LC-MS/MS-metode er udviklet og valideret til påvisning og kvantificering af tilbageværende koncentration af nisin i ost efter forarbejdning/varmebehandling. Yderligere er den anti-listerielle effekt af den tilbageværende nisin-koncentration undersøgt i laboratorie-bouillon samt i ost. En matematisk model er udviklet, som kan beskrive den vækshæmmende effekt af nisin i ost afhængigt af øvrige produktegenskaber inkl. pH. Arbejdet med nisin er sammenfattet i en videnskabelig publikation (reference nr. 5 i sektion 13).

Opgave 5: Prædiktionsværktøj med nye validerede L. monocytogenes modeller til mejeriprodukter.
Syv matematiske modeller er identificeret/kalibreret/udviklet til forudsigelse af vækstpotentialet for *L. monocytogenes* og kuldetolerante pseudomonader i forskellige typer af oste. Disse modeller er programmeret i MS Excel og er medtaget i den nye version af Food Spoilage and Safety Predictor (FSSP) til at forudsige vækst under konstant og dynamisk temperatur, produktsammensætning, mv.

In English:

This project focused on development of mathematical models to predict growth of spoilage and pathogenic bacteria in a broad range of dairy products. The studied products included milk and smear-, veined-, ripened-, fresh-, cream-, processed-, and chemically acidified cheeses. The project was structured in five sub-activities (Tasks). Task 1 below belongs to the total project period whereas task 2-5 belong to the project period with DDRF funding.

Task 1: Identification of a potential hygiene indicator microorganism(s) for *L. monocytogenes* in the dairy environment and cheese based on the analysis of available data and scientific literature.
Results showed that despite the extensive literature studied, no groups/species of microorganisms different from Listeria was identified as suitable indicator organisms to reflect the level of production hygiene for cheese or as index organisms to assess the prevalence of *L. monocytogenes* in cheeses. (Reference # 2 in section 13).

Task 2: Validation and calibration of available *L. monocytogenes* growth models for different cheeses.
Three challenge tests were performed with *L. monocytogenes* in white mould cheese, where milk was inoculated with the bacterium prior to cheese production in the laboratory. In addition to quantifying growth for *L. monocytogenes* and lactic acid bacteria, the products were characterized by changes in temperature, pH, lactic acid and NaCl/water activity during preparation and storage for 30 days. The experiments showed a pH increase from ~ 4.8 (day 1) to ~ 7 (day 30) and a significant growth of *L. monocytogenes* (> 4 log cfu/g) on the surface/rind of cheeses with *Penicillium camemberti* and *Geotrichum candidum.* Significantly lower pH increase and less growth of *L. monocytogenes* was observed for cheese with *Geotrichum candidum*. For white mould cheese, growth and survival of *L. monocytogenes* was predicted with good accuracy using the previously developed growth and growth boundary model of Østergaard et al. (2014). It was crucial to use measured salt concentration and measured changes in temperature, pH and lactic acid concentration to predict the growth of *L. monocytogenes* in white mold cheese.

Additionally, 10 different existing models of *L. monocytogenes* have been tested for their ability to predict the growth and survival of the bacterium in five different types of cheeses. This evaluation of models was based on 319 experiments/curves for growth and survival of *L. monocytogenes*. Existing suitable models were identified for white mould cheese (DL-culture; n = 38) and brine cheese (O-culture; n = 18). For blue mould cheese (veined; n = 37) and ripened cheese (ripened; n = 11), existing models can be calibrated to obtain acceptable predictions. For fresh cheese (n = 109) a single model could not be identified. The results obtained are included in scientific publication (Reference # 6 in section 13).

A growth boundary model for psychrotolerant pseudomonas in cottage cheese with cultured cream dressing, raw milk and heat-treated milk was developed and validated. The model included terms for the effect of temperature, pH, NaCl/a_w, lactic acid, sorbic acid and interaction among all factors (Reference # 1 in section 13).

Task 3: Extended models to contain the growth-inhibiting effect of dairy-specific ingredients.
Two scientific publications have been prepared (Reference # 3 and 4 in section 13). The first publication describes a comprehensive model that makes it possible to predict growth and growth boundary for *L. monocytogenes* in chemically acidified cheeses and in cream cheese with pH down to 4.6. The second publication describes an extended model which contains the growth inhibitory effect of emulsifying salts on the growth potential of *L. monocytogenes* in processed cheese. Both models are flexible tools that can support the development of new products where *L. monocytogenes* cannot grow.

Task 4: Measure and model the anti-listerial effect of bacteriocins in fermented dairy products.
The work with bacteriocins has focused on nisin in processed cheeses. A new LC-MS/MS method have been developed and validated for the detection and quantification of residual concentrations of nisin in cheese after processing/heat treatment. Furthermore, the anti-listerial effect of residual nisin concentrations has been investigated in laboratory broth and in cheese. A mathematical model was developed to describe the growth-inhibiting effect of nisin in cheese depending on other product properties including pH. The work with nisin is summarized in a scientific publication (Reference # 5 in section 13).

Task 5: Prediction tool with new validated *L. monocytogenes* models for dairy products.
Within the project, seven mathematical models have been identified/calibrated/developed to predict the growth potential of *L. monocytogenes* and psychrotolerant pseudomonas in different types of cheeses. These models are programmed in MS Excel and have been included in the new version of Food Spoilage and Safety Predictor (FSSP) for predicting growth under constant and dynamic temperature and product composition, etc.

7. Project aim

DK: Det overordnede mål med projektet var at udvikle et mikrobiologisk prædiktionsværktøj til forudsigelse af vækstpotentialet for *L. monocytogenes* i flere forskellige mejeriprodukter.

UK: The overall objective of the proposed project is to develop a predictive food microbiology tool that allows the growth potential for *Listeria monocytogenes* to be predicted for a broad range of dairy products.

8. Background for the project

Since the removal of the EU quota in 2015 larger amounts of milk have become available in Denmark/Europe. A competitive solution for the excess milk is the production of cheese which has higher revenue than milk. Denmark is the fifth cheese exporter in the world (5.6% of the total world exports) with total revenue of 1.7 billion US$. In addition, the Danish dairy industry is establishing in new markets were consumption exceeds production of dairy products (e.g. Asia, Africa and Middle East). However, some of the new markets may present challenges regarding control of the cold chain and the dairy products formulation might need to be optimized to achieve the desired shelf-life in the intended distribution chains.

Furthermore, in September 2015, the United Nations (UN) announced a goal of halving worldwide food waste and substantially reducing global food loss by 2030 as part of its Sustainable Development Goals (SDG) agenda. For instance, in Europe every year more than 29 million tons of dairy products go to waste with the majority of waste happening in private households (FAO, 2019). Not only does this pose a significant economic and nutritional loss, it also comes at an environmental cost as dairy products have relatively high greenhouse gas emissions (kg CO2/ kg product) (Clune et al., 2017). Therefore, extension of shelf-life has the potential for reducing food waste across the food supply chain. Increased shelf-life may help retailers sell more of a dairy product before it expires. While increasing the open shelf-life will get consumers more time to consume a product prior to its expiration date and in turn reduce food waste. However, this poses a higher risk for potential growth of spoilage or pathogenic bacteria introduce during production or by consumers and thereby the need to reformulate dairy products.

Growth in dairy products of the pathogenic bacteria *L. monocytogenes* and the group of spoilage psychrotolerant pseudomonads have been extensively described in literature. However, information about product characteristics (e.g. pH, NaCl/a_w, organic acids, emulsifying salts, bacteriocins) that most likely affect growth is not reported in most of the studies. Therefore, it is necessary to obtain precise physico-chemical information of dairy products to be able to identify potential preserving parameters that will reduce or prevent growth of *L. monocytogenes* or psychrotolerant pseudomonads. In relation to the safety of dairy products, identification of product characteristics that prevents growth of *L. monocytogenes* is an interesting perspective (EC, 2005) and a possibility for extending the shelf-life or open shelf-life of dairy products. In fact, FAO/WHO and FDA/USDA risk assessment models predicted that the greatest impact for reducing listeriosis will be achieved by preventing growth to high numbers (FAO, 2004; USDA/FDA, 2003). Therefore, reformulating foods so that they retard or do not support growth of *L. monocytogenes* is one of the recommended approaches to reduce listeriosis cases (ILSI, 2005).

The food industry can use durability studies or challenge tests to assess the effect of product recipes and storage conditions on growth of relevant spoilage or pathogenic microorganisms (EC, 2005; Membré and Lambert, 2008; Legan et al., 2009). However, this is a time consuming and expensive approach (Walker, 2000; Zwietering et al., 1996). Exten-

sive and validated mathematical models containing the most important factors affecting growth of spoilage or pathogenic organisms potentially present in dairy products can be used to optimized product formulation. However, none of the available models includes all those factors most likely to affect growth of *L. monocytogenes* or psychrotolerant pseudomonads in dairy products.

9. Sub-activities in the entire project period

	2015		2016				2017				2018				2019	
	8-9	10-12	1-3	4-6	7-9	10-12	1-3	4-6	7-9	10-12	1-3	4-6	7-9	10-12	1-3	4
Task 1	T 1.1			T 1.2				1.3								
Task 2			T 2.1							T 2.2					T 2.3	
Task 3			T 3.1					T 3.2		T 3.3					T 3.4	
Task 4												T 4.1			T 4.2	
Task 5															T 5.1	

T 1.1	Systematic review of literature
T 1.2	Data analysis: meta-analysis and regression modelling
T 1.3	Data analysis and scientific publication preparation
T 2.1	Growth data collection from literature
T 2.2	Challenge tests to collect experimental data and evaluation of existing models performance
T 2.3	Scientific publication preparation
T 3.1	Experimental data collection to develop new terms for phosphate salts for model development and challenge test with processed cheese and cream cheese
T 3.2	Experimental data collection to develop new pHmin-term and challenge test with chamically acidified cheese
T 3.3	Scientific publication preparation
T 3.4	Scientific publication preparation
T 4.1	LC-MS/MS method developemnt and validation to quantified added nisin in cheese and challenge test with cheeses containing added nisin
T 4.2	Scientific publication preparation
T 5.1	Programming of model in Excel and FSSP software development
	Maternity leave

10. Project results

Task 1. Systematic review of the literature showed that *L. monocytogenes* primarily is involved in outbreaks related to smear or fresh cheese. Meta-analysis of *L. monocytogenes* prevalence in different types of European cheeses revealed (i) that the highest mean prevalence is observed in smear cheese and (ii) mean prevalence in cheeses produced with un-pasteurized milk is similar to those produced with pasteurized milk highlighting the importance of post-pasteurization contamination.

Prevalence and concentrations of *L. monocytogenes* in cheeses are low, hence evaluation of potential presence of other index or indicator microorganisms easier to determine or quantify was considered. Results showed that despite the extensive literature studied, no groups/species of microorganisms different from *Listeria* was identified as suitable indicator organisms to reflect the level of production hygiene for cheese or as index organisms to assess the prevalence of *L. monocytogenes* in cheeses. However, this seems of limited practical information as detection methods for *L. monocytogenes* are available (Reference # 2 in section 13).

Task 2. Existing predictive models for *L. monocytogenes* were evaluated for their ability to predict growth in different types of cheeses including smear, veined, ripened, fresh and brined cheeses. Predictions were compared with growth responses of *L. monocytogenes* extracted from literature studies where constant storage temperature and constant product characteristics were assumed. Predicted and observed μ_{max}-values (n= 319) were evaluated by calculation of bias- (B_f) and accuracy (A_f) factor values. B_f-values from 0.85 to 1.11 indicate a good model performance, whereas B_f-values of 1.11-1.43 and 0.87-0.95 correspond to acceptable model performance. B_f-values <0.87 or > 1.43 corresponds

to unacceptable model performance (Ross, 1996). A_f is a measure of the average difference between observed and predicted μ_{max}-values. $A_f > 1.5$ indicates an incomplete model or systematic deviation between observed and predicted μ_{max}-values (Mejlholm and Dalgaard, 2013). Two models were identified as being able to accurately predict growth of *L. monocytogenes* in smear cheese and brined cheese, respectively (Table 1). Challenge tests were performed to collect *L. monocytogenes* growth data and dynamic product characteristics for smear cheese during production, ripening and storage (Fig. 1). The model of Østergaard et al., (2014) developed to predict growth of *L. monocytogenes* in cottage cheese with cultured and fresh cream dressing was validated for smear cheese by using data collected from the challenge tests (Fig. 2) and from literature (Table 1).

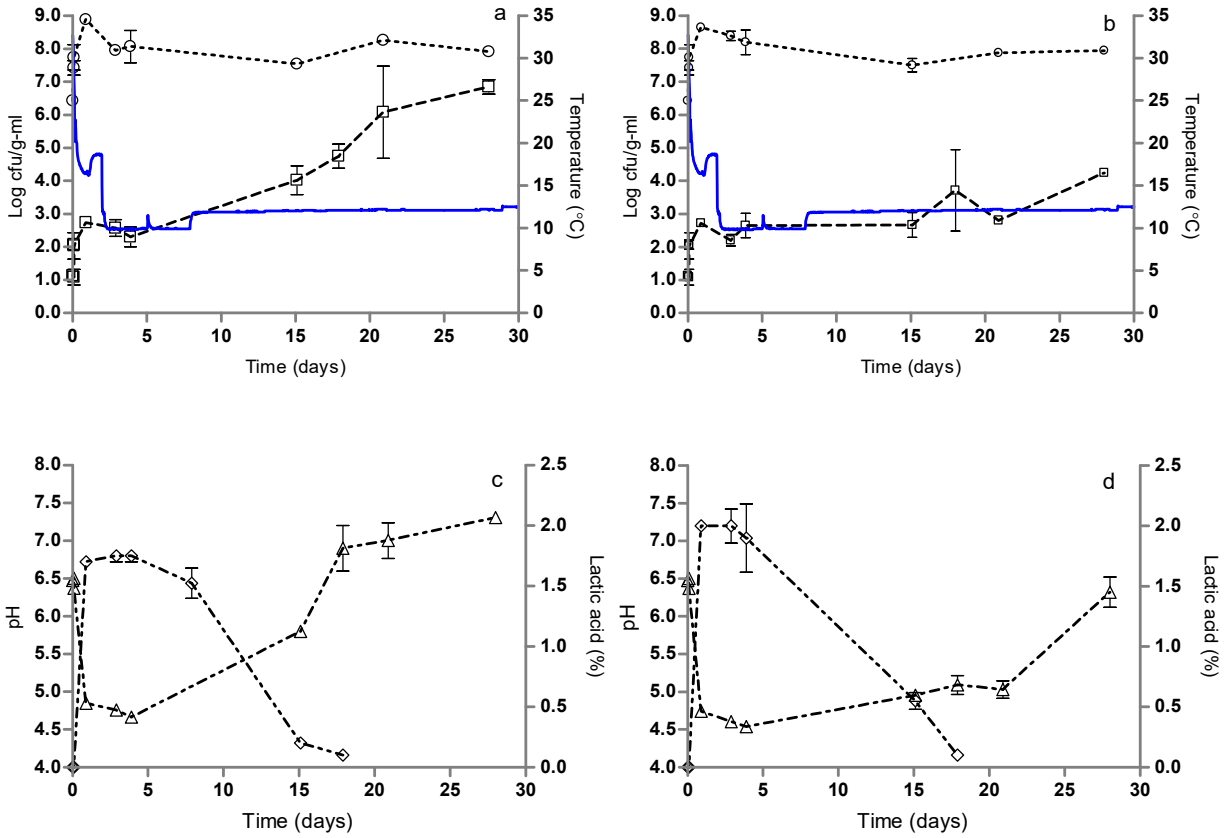

Figure 1. Data for the rind (a, c) and the core (b, d) of smear cheese. *L. monocytogenes* (□) and lactic acid bacteria (○) behaviour in smear cheese with *Lactobacillus acidofilus*, LA-5 and FLORA™ C501, Chr. Hansen A/S, *Penicillium camemberti*, PC HP 6 LYO and *Geotrichum candidum*, GEO 17, DuPont™ Danisco®. Evolution of pH (△) and lactic acid concentration in % (◇). The temperature profiles are shown as blue lines in the top figures. Symbols represent average values and error bars of the standard deviation for three samples.

A growth and growth boundary model for psychrotolerant pseudomonads in cottage cheese with cultured cream dressing and milk was developed and validated. This model including the effect of temperature, pH, NaCl/aw, lactic acid, sorbic acid and interactions can be used to optimized cottage cheese formulations that will prevent psychrotolerant pseudomonads from reaching the minimal spoilage level during the intended shelf-life (Reference # 1 in section 13).

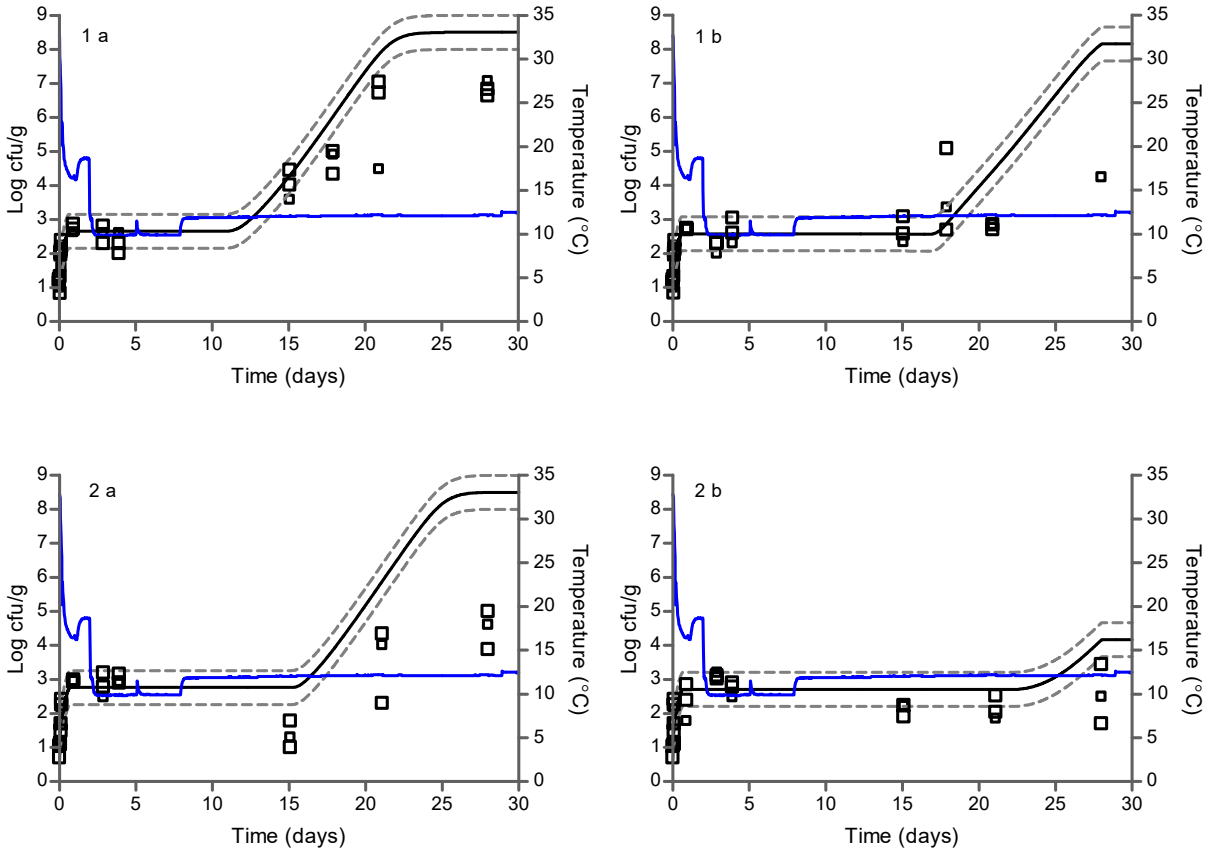

Figure 2. Comparison of observed (□) and predicted (-) growth of *L. monocytogenes* in the rind (a) and core (b) of two challenge tests with smear cheese. Solid lines represent the predicted growth by the model of Østergaard et al., (2014). Graphs include the acceptable simulation zone (±0.5 Log cfu/g, dashed lines).

Table 1. Comparison of observed (literature data) and predicted maximum specific growth rates (μ_{max}-values) of *L. monocytogenes* in different types of cheese

Type of cheese	G[a]	Bias factor / Accuracy factor values for the ratio between predicted and observed growth rates									
		Østergaard et al.. (2014) (μ_{ref}=0.34)[b]	Østergaard et al. (2014) (μ_{ref}=0.72)[c]	Augustin et al. (2005) (μ_{opt}=0.742)[d]	Martinez-Rios et al. (2019)	Sym'Previus	Augustin et al. (2005) (μ_{opt}=0.212)[e]	Schvartzman et al. (2011) (μ_{opt}=1.19)[f]	Schvartzman et al. (2011) (μ_{opt}=0.17)[g]	Rosshaug et al. (2012)	ComBase
Smear	38	**1.0/1.5**	2.1/2.2	1.4/1.7	1.4/1.6	1.8/2.0	0.4/2.6	2.2/2.4	0.3/3.3	2.1/2.2	2.2/2.2
Veined	37	1.3/2.4	2.7/3.3	2.3/2.9	1.7/2.6	3.5/3.7	0.6/2.0	3.0/3.2	0.6/1.9	3.0/3.0	2.9/3.2
Ripened	11	0.6/2.5	1.2/1.8	0.5/2.8	0.7/2.3	1.0/1.9	0.1/7.3	0.8/2.2	0.1/9.3	1.2/1.8	0.9/2.0
Brined	18	0.6/2.1	**1.2/1.6**	0.8/1.8	0.7/1.9	**1.1/1.6**	0.2/4.4	1.3/1.9	0.2/5.5	1.3/1.8	1.3/1.7
Fresh	109	0.7/2.4	1.4/2.2	1.0/2.3	0.9/2.2	0.9/2.9	0.3/4.2	1.6/2.5	0.2/5.0	0.8/3.9	1.4/2.2
Total	213	0.8/2.2	1.6/2.2	**1.1/2.3**	**1.1/2.1**	1.3/2.6	0.3/3.7	1.7/2.6	0.2/4.4	1.1/3.2	1.7/2.3

[a] G, number of experiments where growth was observed
[b] Østergaard et al., 2014, aroma culture (DL-culture)
[c] Østergaard et al., 2014, no-aroma culture (O-culture)
[d] Augustin et al., 2005, liquid dairy products
[e] Augustin et al., 2005, cheese
[f] Schvartzman et al., 2011, pasteurized milk cheese
[g] Schvartzman et al., 2011, raw milk cheese

Task 3. The effect of temperature on the minimum pH that supports growth of *L. monocytogenes* (pH_{min}) was quantified in broth studies and by using literature data obtained with different pH values and different constant temperatures. A model was developed to describe the effect of temperature on the minimum pH for *L. monocytogenes* growth. A growth and growth boundary model was developed by substituting the constant pH_{min}-value present in an existing model by the new pH_{min}-term. Challenge tests where *L. monocytogenes* was inoculated in chemically acidified cheese (glucono-delta-lactone; GDL) and cream cheese were performed to collect growth responses in low pH foods. In addition, literature data for growth of *L. monocytogenes* in products with or without GDL were collected. Growth rates of *L. monocytogenes* were accurately predicted by the new model in a broad range of foods. Growth and no-growth responses of *L. monocytogenes* in seafood, meat, non-fermented dairy products as well as fermented cream cheese were 90.3% correctly predicted with the incorrect predictions being 5.3% fail-safe (growth predicted when no growth was observed) and 4.4% fail-dangerous (no growth predicted when growth was observed). The new model can support product development, reformulation or risk assessment of a wide variety of foods including meat, seafood and different dairy products (milk, cream, desserts, chemically acidified cheese and cream cheese) with pH-values as low as 4.6.

The antimicrobial effect of phosphate salts necessary to produce spreadable processed cheese was examined in broth studies and their inhibiting effect on *L. monocytogenes* growth was modelled. It was concluded that emulsifying salts can be used as an additional growth hurdle in spreadable processed cheese in order to prevent growth if the pathogen e.g. is introduced by consumer handling after opening the hot-filled package. A mathematical model was developed to predict the effect of phosphate salts, lactic acid, acetic acid, citric acid, pH, aw, temperature and interactions amongst all these factors on growth and the growth boundary of *L. monocytogenes* in spreadable, processed cheese. Challenge tests showed that both growth and the growth boundary were accurately predicted by the developed model. The growth and growth boundary model correctly predicted 54 of 60 growth and no growth responses of *L. monocytogenes* in spreadable processed cheese. The developed model can be used by the dairy sector to facilitate formulation of safe recipes and this approach seems faster and more cost effective than the traditional challenge testing.

Task 4: The model described in the previous paragraph was further expanded to contain a term to account for the inhibiting effect of nisin A added as preservative to processed cheese. The antimicrobial activity of nisin A against *L. monocytogenes* was quantified in broth studies and additional antimicrobial data, obtained at different pH-values, were collected from the literature. A nisin-term was developed to describe the effect of pH on the antimicrobial activity of nisin A (Fig. 3). Furthermore, a liquid chromatography/mass spectrometry (LC-MS/MS) method was developed and validated to quantify nisin A and Z present in cheese. Challenge tests were performed to generate data for model evaluation. When the quantified residual nisin A concentrations measured by LC-MS/ MS in cheese were used as model input this resulted in accurate predictions of growth for *L. monocytogenes*. However, if the added concentration of nisin A was used as model input, the model markedly underestimated growth. The model can support risk assessment and product development, but further studies with higher residual concentrations of nisin A in cheeses will be beneficial for model validation.

Task 5. The models identified/calibrated/developed in this project to predict the growth potential of *L. monocytogenes* and psychrotolerant pseudomonas in different types of cheeses have been programmed in MS Excel to and the models are being included the new version of the Food Spoilage and Safety Predictor (FSSPv5) software to facilitate their use by industry, authorities and scientists.

Conclusions:

This project has demonstrated that accurate prediction of *L. monocytogenes* and psychrotolerant pseudomonads growth in different types of cheeses is possible when using extensive models containing all relevant intrinsic/extrinsic factors and products are fully physico-chemically characterized.

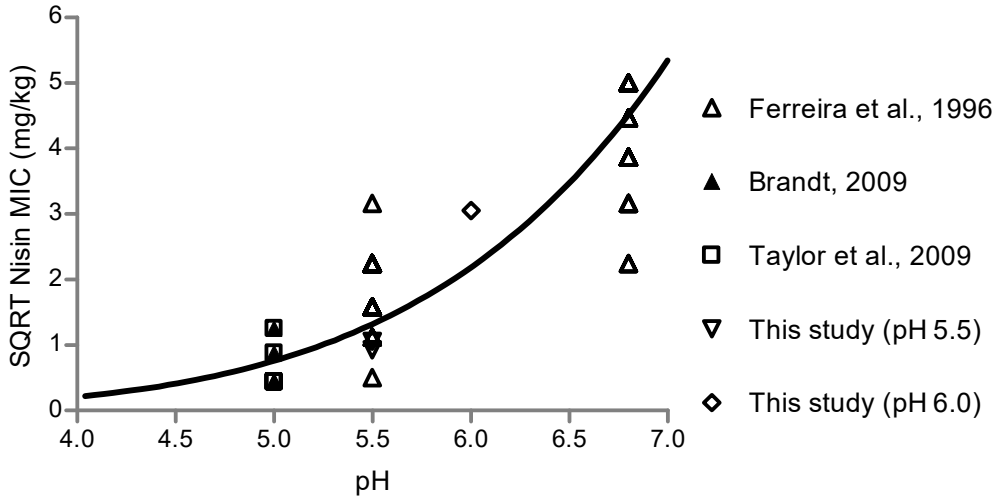

Figure 3. Effect of pH on nisin A minimum inhibitory concentration values for *L. monocytogenes* in broth media.

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11. Deviations

The PhD student employed on this project Veronica Martinez-Rios was on maternity leave during the period including July 2016 - February 2017 and the project has been extended accordingly. Apart from this time-shift in the project period the project has been on track with no deviations related to scientific matters, financial aspects or timetable.

12. The relevance of the results, including relevance for the dairy industry

Scientific relevance

- Systematic review of the literature identified smear- and fresh as the cheese types primarily involved in listeriosis outbreaks.
- Highest mean prevalence of *L. monocytogenes* was observed in smear cheese.
- Mean prevalence of *L. monocytogenes* in cheese produced with un-pasteurized milk is similar to those produced with pasteurized milk highlighting the importance of post-pasteurization contamination.
- Two existing models were identified as being able to accurately predict growth of *L. monocytogenes* in smear cheese and brined cheese.
- A model was developed to predict growth of psychrotolerant pseudomonads in cottage cheese with cultured cream dressing

- A new pH_{min}-term was developed that describes the effect of temperature on the minimum pH that supports growth of *L. monocytogenes.*
- A growth and growth boundary model with the ability to accurately predict growth of *L. monocytogenes* in a wide variety of foods including meat, seafood and different dairy products (milk, cream, desserts, chemically acidified cheese and cream cheese) with pH-values as low as 4.6 has been developed and validated.
- A growth and growth boundary model for *L. monocytogenes* in spreadable processed cheese and processed cheese containing nisin has been successfully developed and validated.
- An LC-MS/MS method to quantified concentrations of residual nisin A and Z in processed cheese was developed and validated.

Relevance for the society

Models developed in this project can support risk assessments, product development or reformulation of existing products in order to reduce or inhibit growth of *L. monocytogenes* resulting in safer products for consumers. The product reformulation can contribute to extend shelf-life of dairy products in the market and consequently reduced food waste.

Relevance for the industry

The dairy sector can reduce the number of durability studies or challenge tests resulting in faster product development and reduced time-to-market for new or modified products.

Future projects

- Effect of heat treatment for processed cheese on residual nisin concentrations in products

It appears interesting to evaluate and model the effect of the heat treatment (temperature and time) used to produce processed cheese on the residual concentrations of nisin A. Following results obtained in Task 4, it is clear that there is a big discrepancy between the concentration of nisin added to processed cheese before heat treatment and the residual nisin concentration quantified by LC-MS/MS. Furthermore, it seems interesting to compare residual nisin A quantification results obtained with LC-MS/MS and results from the extensively used agar diffusion methods used to quantify nisin activity.

13. Communication and knowledge sharing about the project

Papers in international journals:

1. Martinez-Rios, V., Østergaard, N.B., Gkogka, E., Rosshaug, P.S., Dalgaard, P. (2016). Modelling and predicting growth of psychrotolerant pseudomonads in milk and cottage cheese. *International Journal of Food Microbiology* 216, 110-120.

2. Martinez-Rios, V., Dalgaard, P. (2018). Prevalence of *Listeria monocytogenes* in European cheeses: A systematic review and meta-analysis. *Food Control* 84, 205-214.

3. Martinez-Rios, V., Gkogka, E., Dalgaard, P. (2019). New term to quantify the effect of temperature on pH_{min}-values used in cardinal parameter growth models for *Listeria monocytogenes*. *Frontiers in Microbiology* 10:1510. doi: 10.3389/fmicb.2019.01510

4. Martinez-Rios, V., Jørgensen, M.Ø., Koukou, I., Gkogka, E., Dalgaard, P. (2019). Growth and growth boundary model with terms for melting salts to predict growth responses of *Listeria monocytogenes* in spreadable processed cheese. *Food Microbiology*. 84, 103255. https://doi.org/10.1016/j.fm.2019.103255.

5. Martinez-Rios, V., Pedersen, M., Pedrazzi, M., Gkogka, E., Smedsgaard, J., Dalgaard, P. Cardinal parameter model containing nisin term to predict growth of *Listeria monocytogenes* in processed cheese. Manuscript in preparation for special issue of *International Journal of Food Microbiology*.

6. Martinez-Rios, V., Gkogka, E., Dalgaard, P. Evaluation of predictive models for growth of *Listeria monocytogenes* in cheeses. Manuscript in preparation for International Journal of Food Microbiology.

Easily read papers:

Kjer, U. (2018). Ny software kan forudsige listeria i oste. http://www.mejeri.dk/nyheder/2018/ny-software-kan-for-udse-listeria-i-oste.

Dalgaard, P., Martinez-Rios, V. (2019). Prædiktionsværktøj til produktudvikling og risikovurdering. Mælkeritidende, No. 12, side 14. https://maelkeritidende.dk/sites/default/files/udgivelser/3005625_mt_12_2019_forskning-1.pdf

Student theses:

Veronica Martinez-Rios PhD tesis (2019). New models for safety and quality assessment of a broad range of dairy products.

Marie Østergaard Jørgensen MSc thesis (2016). Prediction of growth of *Listeria monocytogenes* in processed and cream cheese.

Sarah Samir Kadhim MSc thesis (2018). Prediction of *Listeria monocytogenes* growth in chemically acidified white cheese.

Monica Pedrazzi MSc thesis (2018). Model to predict growth and survival of *Listeria monocytogenes* in cheese with added nisin.

Oral presentations at scientific conferences, symposiums etc.:

Martinez-Rios, V., Pedersen, M., Pedrazzi, M., Gkogka, E., Smedsgaard, J., Dalgaard, P. (2019). Cardinal parameter model containing a new nisin term to predict growth of Listeria monocytogenes in processed cheese. Abstract for oral presentation at 11th International Conference on Predictive Modelling in Food, 17-20 September 2019, Braganza, Portugal.

Martinez-Rios, V. Predictive microbiology – converting predictions into practice. **Invited speaker** at Dairy Research Day on 27th March 2019, Billund.

Dalgaard, P., Martinez-Rios, V., Koukou, I. Cowan, B.J. (2018). FSSP – Food Spoilage and Safety Predictor. Oral presentation and software demonstration at ICPMF Software Fair for Predictive Microbiology and Risk Assessment Tools, FoodMicro 2018 – 26th International ICFMH Conference, 3-6 September 2018, Berlin, Germany.

Martinez-Rios, V., Jørgensen, M. Ø., Koukou, I., & Dalgaard, P., 2018. Expanded cardinal parameter model with terms for phosphates salts to predict growth of *Listeria monocytogenes* in spreadable cheeses. **Poster presentation** at the 26th International ICFMH Conference – FoodMicro 2018, Berlin.

Martinez-Rios, V. & Dalgaard, P. New term for the effect of temperature on pH_{min}-values in cardinal parameter growth models for *Listeria monocytogenes*. **Poster presentation** at the 26th International ICFMH Conference – FoodMicro 2018, Berlin.

Martinez-Rios, V., Koukou, I., Jørgensen, M.Ø., Kadhim, S., & Dalgaard, P., 2017. Predictive microbiology for the dairy industry. **Invited speaker** at the Danish Society of Dairy Technology shelf-life seminar on 7th December, 2017.

Dalgaard, P., Koukou, I., Martinez-Rios, V. (2017). Predictive modelling to improve and document safety of dairy products. Invited presentation at Nordic Dairy Congress, 7-9 June 2017, Copenhagen, Denmark.

Martinez-Rios, V. & Dalgaard, P., 2017. Prevalence of *Listeria monocytogenes* in European cheese: A systematic review and meta-analysis. **Speaker** at the 44th Nordic Dairy Congress, 2017, Copenhagen.

Martinez-Rios, V., Østergaard, N.B., Rosshaug, P., Dalgaard, P. (2015). Modelling and predicting growth of psychrotolerant pseudomonads in milk and cottage cheese. Abstract and poster presentation at DMS Kongres 2015, 9 November 2015, Copenhagen, Denmark.

Oral presentations at meetings:

Martinez-Rios, V. Dairy-PREDICT mid-term project results at Arla Innovation Centre on 8th December 2017.

Presentations at DDRF steering group meetings.

14. Contribution to master and PhD education

A PhD student, Veronica Martinez-Rios (see section 13, student theses).

A 45 ECTS MSc-project and two 30 ECTS MSc-projects were developed as part of master student studies (see section 13, student theses). The students were trained in the methodology required to develop new terms for a model and challenge testing performance along with model development and evaluation.

15. New contacts/projects

The project has established collaboration between Arla Foods and DTU Food. This collaboration has resulted in a one year Postdoc project and we believe it will become a highly valuable collaboration for future developments in the area of predictive dairy microbiology.

16. Signature and date

The project is formally finalised when the project manager and DDRF-representative (e.g. steering committee leader) have signed this final report.

Date: 12 July 2019; Signature, Project manager:

Date: 12 July 2019; Signature, DDRF-representative: