SLUTRAPPORT
NR. 2018-154

Et nyt paradigme inden for osteproduktion – anvendelse af cellular automaton modellering til reduktion af ostemodningstiden

Mejeribrugets
ForskningsFond

JUNI 2018

Final report

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

1. Title of the project

Et nyt paradigme indenfor osteproduktion - anvendelse af cellular automaton modellering til reduktion af ostemodningstiden.

A new paradigm in cheese processing – using cellular automaton modelling for reduction of cheese ripening time

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4. Sources of funding

Danish Dairy Research Foundation (Mælkeafgiftsfonden/Milk Levy Fund) and Innovation Fund Denmark (Højteknologifonden).

5. Project period

Project period with DDRF funding:

Start 1/2015

End 12/2017

6. English project summary

Overall, the project has been running according to the plan. However, minor adjustments have been made based on the results obtained.

The project consisted of three work packages:

In **work package 1**, a new model to monitor growth of starter bacteria (SLAB) and non-starter bacteria (NSLAB) has been developed. The most important result is that diffusion has very limited significance for the growth of NSLAB and consequently for the maturation of cheese. Diffusion will only have an effect in the rare cases where the growth and maturation potentials of NSLAB depend on very large molecules. So far, nothing indicates that this should be the case.

We embarked on the task to model aroma formation in cheese. The literature available is extensive. The conclusion was that modelling of aroma formation kinetics would be rather senseless due to the lack of quantitative data. The literature contains a vast number of references on qualitative data indicating that many thousand different aroma processes are taking place simultaneously in the cheese. It is impossible to identify the ones that will be most significant in terms of flavor. Consequently, creating a meaningful model for aroma formation will be extremely difficult.

In **work package 2**, a fully operational pilot plant facility for cheese production was established. Experiments were made to study the effect of different sugars on the growth of NSLAB. The ability to initially boost the growth of NSLAB may affect the speed of maturation (as also shown in our modelling simulations). The result was somewhat depressing and unexpected as none of the tested sugars had any effect on the growth of the NSLAB. Later, it became clear that the maturation in this particular experiment did not run as it usually would. NSLAB did not establish itself in the cheese, and the autolysis of SLAB was much slower than normally seen during maturation. A possible explanation could be that the cheese experiments were carried out using a brand-new cheese pilot plant, where NSLAB had not yet established itself as part of the production environment.

Initial lab experiments showed that NSLAB were able to grow on lysed SLAB cells. This indicates, as expected from the model, that the lysed SLAB cells may be used by the NSLAB to grow in the cheese matrix.

In **work package 3**, a method used to determine diffusion of amino acids and peptides was developed. A model used to fit data and calculate diffusion rates was established. Interestingly, diffusion in a normal

cheese turned out to be significantly slower than what has been shown in previous studies measuring diffusion in model systems. Actually, the diffusion of peptides was up to ten times slower than the diffusion of far bigger molecules, measured in model systems.

6. Dansk projektsammendrag

Projektet har i det store og hele fulgt projektplanen. Dog har ny viden, genereret undervejs i studiet, ført til adskillige justeringer i prioriteringen af de enkelte punkter i planen.

Projektet har bestået af tre arbejdspakker:

I **arbejdspakke 1** er der udviklet en model til at følge vækst af starterbakterier (SLAB) og non-starter bakterier (NSLAB). Det overordnede **resultatet er, at diffusion har meget begrænset betydning for vækst af NSLAB og dermed også for modning af ost.** Kun hvis NSLABs vækst og evne til a modne osten afhænger af endog meget store molekyler, kan diffusion få en betydning. Indtil videre er der ikke noget der indikerer, at dette skulle være tilfældet.

Vi har igangsat arbejde med at modellere aromadannelse i ost. Litteraturarbejdet har været meget omfattende. Konklusionen er, at der ikke findes tilstrækkelig kvantitative data til meningsfuldt at kunne modellere aromadannelseskinetik i ost. Til gengæld er der en omfattende litteratur af kvalitative data, som indikerer at flere tusinde aromaprocesser foregår samtidigt i osten. Hvilke der er de smagsbestemmende er pt. umuligt at afgøre, hvorfor det også er meget svært at lave en meningsfuld model for aromadannelse.

I **arbejdspakke 2** er der etableret en fuldt fungerende pilotskala-anlæg til produktion af ost. Der blevet etableret en forsøgsrække, der gør det muligt at studere effekten af forskellige sukkerstoffers betydning for vækst af NSLAB. Kan man i den initiale fase af modningen booste væksten af NSLAB kan dette have betydning for modningshastigheden (dette ser vi også i vores modelsimuleringer). Resultatet er lidt nedslående, da ingen af de sukkerarter, der har været testet, ser ud til at have den effekt på vækst af NSLAB, der var forventet. Det har efterfølgende vist sig, at modningsprocessen ikke har forløbet helt, som det normalt sker. NSLAB ser ikke ud til at have etableret sig i osten, og autolysen af SLAB har været væsentligt langsommere end det normalt ses under ostemodning. En forklaring kan være, at osteforsøgene er foregået i et helt nyt oste-pilotanlæg, hvor NSLAB endnu ikke har fået lov at etablere sig i produktionsmiljøet.
Indledende laboratorieforsøg viser, at NSLAB kan gro på lyserede SLAB celler. Dette indikerer, som antaget i modellen, at lyserede SLAB celler kan være en væsentlig kilde til vækst af NSLAB i ostematricen.

I **arbejdspakke 3** er en metode til at bestemme diffusion af aminosyre og peptider fuldt udviklet. Der er etableret en model til at ”fitte” data og beregne diffusionshastigheden. Resultaterne viser, meget interessant, at diffusion i en normal ost ser ud til at være væsentligt langsommere end målt i tidligere studier, hvor diffusion er målt i modelsystemer. Faktisk er diffusion af peptider helt op til 10 gange langsommere end diffusionen af langt større molekyler målt i modelsystemer.

7. Project aim

Målet med dette projekt er at udvikle en ny metode/nyt paradigme inden for ostemodning, hvor vi kombinerer matematisk modellering baseret på såkaldt "cellular automation"-principper med udvikling af specifikke laboratoriemetoder, der gør det muligt at minimere den tid, der bruges til ostemodning uden at kompromittere ostens smag og tekstur.

The aim of this project is to establish a new paradigm in cheese processing combining a mathematical modelling approach based on the principles of "cellular automaton" and well-defined laboratory experiments to determine the minimal ripening time without compromising quality (taste and texture).

8. Background for the project

The cheese microbiota is pivotal to nearly all processes taking place during cheese production. Together with the rennet, the growth of the starter bacteria and conversion of lactose to lactate are causing the milk to coagulate. The starter bacteria are also important for degradation of casein peptides and formation of aroma compounds, a process which together with the salt content influences water activity and thus also the growth conditions for the individual bacteria as well as the diffusion of e.g. nutrients, peptides and flavor compounds (for a review see e.g. Bereford et al. 2001).

There is today – estimated – bound approx. 2 billion. DKK in cheeses in storage that are ripening or waiting to be sold. If one is able to optimize the ripening time, for example by cutting 10% off the ripening period, it will be possible to release capital that can be used for new investments. In addition, if the storage time is reduced, this will also mean that less energy needs to be used for heating respectively cooling of the ware houses, which will also mean reduced emission of CO_2 per kg.

During ripening, the microorganisms grow as immobilized colonies in the cheese, making them dependent on diffusion of metabolites in the cheese matrix. The distribution of the immobilized bacteria cells in the cheese matrix is random and the mean distance between the colonies is strongly affected by the initial inoculation level (Jeanson et al., 2011). The diffusion of salt through the cheese matrix has been extensively studied, whereas surprisingly little work has been done on diffusion of other small solutes e.g. small peptides and aroma compounds (Floury et al., 2010). Fluorescence labelled dextran and whey proteins have been used to follow the diffusion of these molecules using fluorescence recovery after photobleaching (FRAP) in a UF model cheese system (Silva et al., 2013). The results showed that that both the size and the charge of molecules are important for their ability to diffuse in the cheese. However, more research is required to understand the importance of diffusion of essential metabolites during cheese ripening.

Taken together, the right timing and rate of growth and autolysis of starter bacteria is crucial for optimal cheese production. On one hand optimal degradation of peptides is important to prevent presence of too many off flavors/bitter peptides and on the other hand, if one wants to accelerate ripening, autolysis should be initiated as fast as possible to support the growth of non-starters producing the essential flavor compounds. Furthermore, the right inoculum size of the starter bacteria should be balanced to create the right size and timing of autolysis as well as the right balance and distance between the starter colonies and the non-starter bacteria to support optimal growth of non-starters and production of the right flavor compounds.

In this study, we intended to couple a stochastic mathematical model with diffusion to address and understand the three-dimensional spatial interplay between limits of growth and autolysis of the starter culture and its subsequent contribution to growth and flavor formation of the non-starter bacteria. The cellular automaton model consists of a grid of cells that may be occupied with bacteria, and in which growth and autolysis according to a set off rules that will be adjusted to diffusion of growth-limiting nutrients.

In order to develop the mathematical model and estimate optimal conditions for cheese ripening, input data about bacterial growth, autolysis rates and diffusion rates of peptides, amino acids and aroma compounds are required under various physical and biochemical conditions. In the study, we intended to design a set of well-defined methods that will enable the cheese producer to obtain these data.

9. Sub-activities in the entire project period

		2015				2016				2017			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Work-package 1	Establishement of cellular automaton based 3D model for growht of starters in a cheese matrix				D1.1								
	Establishement 3D model for non-starter growth in a cheese matrix						D1.2						
	Modelling flavor formation, texture changes etc								D1.3				
	Merging of models										D1.4		
Work-package 2	Establishment of pilot scale cheese production set'up			D2.0									
	Develop method to investigate starter bacteria colony growth in a cheese matrix				D2.1								
	Development of method to investigate starter culture autolysis rates							D2.2					
	Development of method to investigate growth of non-starter cultures										D2.3		
Work-package 3	Establishment of FRAP based method to determine diffusion of metabolites in cheese				D3.1								
	Investigation of diffusion of different metabolites at differnt physiological conditions								D3.2				
	Exploring alternative methods to FRAP for a more direct method to determine diffusion											D3.3	

D1.1 – Model for growth and autolysis of starter bacteria
D1.2 – Model is added growth of non-starters
D1.3 – Model is added degradation and diffusion of peptides and formation and diffusion of flavor compounds
D1.4 – Scenario analysis have been carried out to define the most optimal conditions for ripening

D2.0 - Pilot scale cheese production set'up established
D2.1 - Method to determine colony formation rate and colony sizes of starter bacteria
D2.2 - Method to determine autolysis rates of starter bacteria
D2.3 - Method to determine growth of non-starter bacteria as function of distance to starter bacteria.

D3.1 - Method to determine diffusion coefficients in cheese of molecules with different size and charge.
D3.2 - Determination of diffusion rates under different physiological conditions and in different cheese compartments
D3.3 - Feasibility of alternative and more direct methods, to determine diffusion coefficients in cheese.

10. Project results

Work package 1:

Modelling colony formation and diffusion

In WP1, a stochastic model was developed to follow the development of NSLAB colonies, decay of SLAB and diffusion of nutrients in Cheddar cheese. Initially, a 3D model based on cellular automaton processes was considered, but initial tests on the model showed that a 2D model was sufficient to describe the growth, autolysis and diffusion phenomena of relevance during cheese ripening. Therefore, we ended up making a 2D model instead of a 3D model.

Our modelling studies showed that based on existing literature on diffusion rates of metabolites in cheese-like structures, diffusion of even rather large molecules cannot be a limiting factor for ripening/NSLAB growth in relatively water-rich cheeses such as Cheddar, Gouda, and Danbo.

Even though our own studies of diffusion rates of peptides (WP3) have proven to be significantly lower in the Cheddar cheese than previously indicated in cheese-like structures, the conclusions of the model still hold. Diffusion of small to medium-size macromolecules is not the bottleneck of the ripening process, because SLAB decay supply nutrients at a much lower rate than diffusion could forward them to the immobilized NSLAB colonies. This applies even to be the case for fast-lysing SLAB cultures. The differences of the expected NSLAB growth curves due to differences in nutrient diffusion are small within the realistic range of nutrient diffusion rates (2.0000 - 0.0020 mm^2/hour; the upper four green curves in Fig.1). The model and results were recently published in the International Dairy Journal.

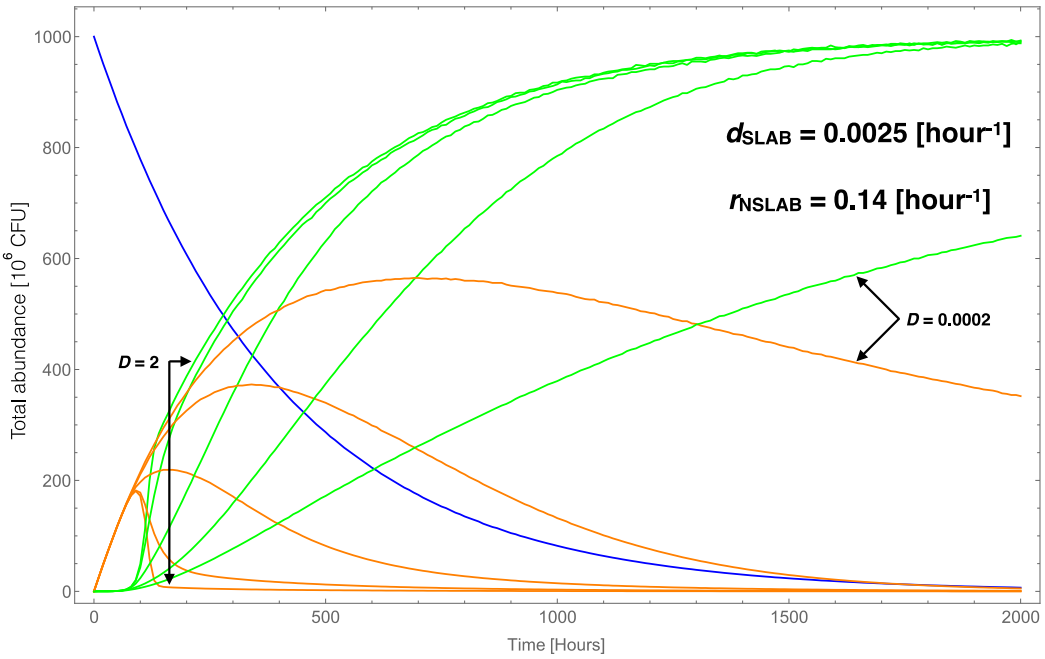

Figure 1: Simulated time courses of SLAB decay, nutrient concentration and NSLAB growth during the secondary ripening period, at SLAB decay rate d_{SLAB} = 0.0025 1/hour and NSLAB growth rate r_{NSLAB} = 0.14 1/hour. These parameter values correspond to the technologically feasible situation of using a fast lysing SLAB culture and ripening at about 10 °C. Green curves: total NSLAB density $\bar{N}(t)$; ochre curves: total nutrient concentration $\bar{F}(t)$, at different nutrient diffusivities (D = 2.0000, 0.2000, 0.0200, 0,0020, 0.0002 mm^2/hour)

In total, the simulations studies made us conclude that it is not diffusion of metabolites that is limiting for the rate of ripening, but rather the rate by which NSLAB species develop during ripening, which may also be closely linked to the decay rate of SLAB, since lysed SLAB may function as a source of nutrients for the NSLAB.

Our results also led to a significant change of focus in WP 2. Instead of focussing on colony formation and initial inoculum size of NSLAB, it was decided to focus research towards methods that could potentially speed up the initial growth of NSLAB. Two lines of studies were decided: i) can we speed up NSLAB growth through an alternative source of sugar? and ii) can we speed up NSLAB growth by adding lysed SLAB cells?

Flavor modelling

A detailed literature study has been carried out with the aim to establish a flavour model. Unfortunately, and to our surprise, there is a huge lack of quantitative data on flavour formation. Qualitative data clearly show that there are literally thousands of different catabolic reactions involved in cheese ripening. In principle, we can establish a model for flavour formation, however, without any quantitative data to indicate which of the biochemical pathways are the most important for ripening we decided not to pursue this path any further.

Instead the modelling capacity was used in WP3 to create a model that could fit our FRAP-based diffusion data.

Work package 2

As explained above our modelling studies in WP1 clearly indicated that NSLAB must be a key determinant to increase ripening time. Therefore, instead of focusing on the SLAB autolysis process (deliverable D2.2), we have focused on deliverable D2.3, developing methods to study and enhance growth of NSLAB in the cheese.

Initially a new cheese pilot plant was established, and shortly after it was decided to initiate a cheese trial to determine NSLAB growth of different sugars.

Eight Cheddar cheeses were manufactured in order to evaluate the effect of the selected sugars (N-acetyl-glucosamine, Ribose and N-acetylgalactosamine) on growth of non-starter lactic acid bacteria. From September 14th 2016 to March 14th 2017, the control cheeses (with or without amino acids) were compared every month in relation to the cheeses with the selected sugars, tested individually or in combination with amino acids (used to accelerate the possible effect of sugars). Levels of salt in moisture and pH were mostly within the range expected for high quality cheeses. However, no differences were shown in the bacterial levels (Figure 2) extracted from any of the eight cheeses, during the six months ripening period at 10° C.

What was expected to be NSLAB started to establish on the MRS selective media after 4-8 weeks and remained at relatively high levels in the remaining ripening period. In total 20 colonies picked from the MRS plates were isolated and analyzed by 16sRNA sequencing. To our surprise, all the strains appeared to be SLAB strains that in somehow adapt to the MRS conditions during the ripening process. Another surprising observation was that the SLAB cells did not autolyze to the extent, which had previously been reported in similar studies.

Finally, it was also noted that of the sugars, N-acetylgalactosamine and ribose, only ribose was slowly degraded, while N-acetylgalactosamine remained undegraded throughout the study.

The conclusion is that the cheese did not follow the usual ripening path, mainly because NSLAB did not establish in the cheese system. An explanation could be that the pilot plant is so new that an NSLAB flora has not yet been established in the production environment. Interestingly though, the lack of NSLAB flora seems to have a significant influence on the rate of SLAB autolysis. The lack of the NSLAB flora could also explain why the degradation of added sugars is so low during ripening, because SLAB cannot degrade the added sugars. A manuscript has been prepared for a short communication in International Dairy Journal.

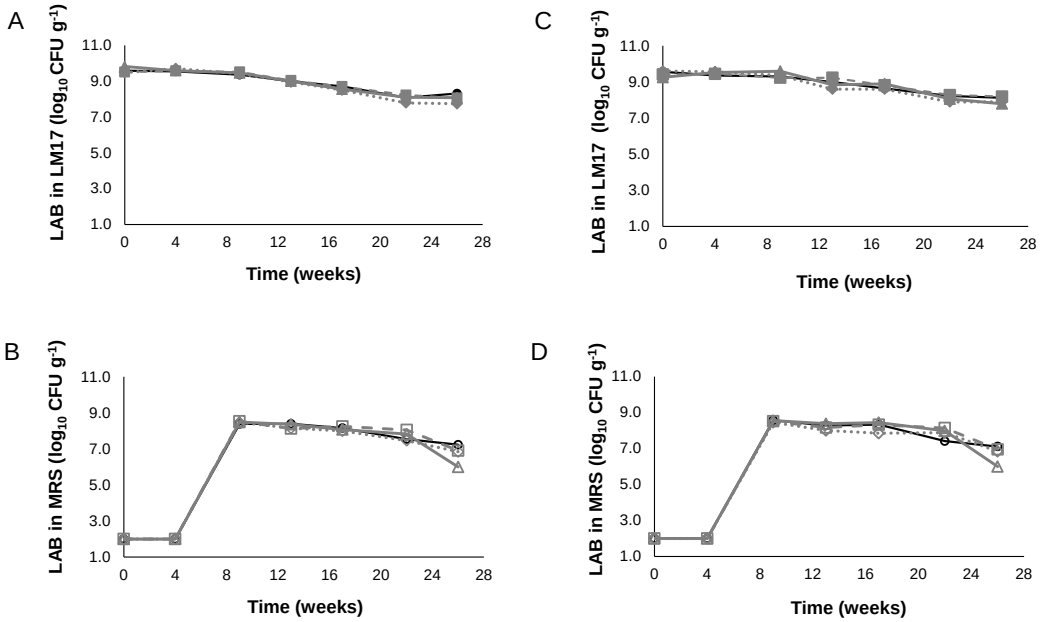

Figure 2 Changes in levels (log^{10} CFU g^{-1}) of lactic acid bacteria, under aerobic conditions on LM17 agar (closed symbols) and under anaerobic conditions on MRS (open symbols), in cheeses with no addition of sugars (in black, ○) or in cheeses made with addition of sugars (in grey): N-Acetylglucosamine (Δ), Ribose (◇) or N-Acetylgalactosamine (□). Vacuum packaged cheeses without (A, B) or with (C, D) casamino acids were ripened at 10° C for six months.

More recently, studies were also carried out to determine the growth of NSLAB strains on lysed SLAB cells. A protocol to obtain full breakdown of a commercial starter culture by enzymatic digestion was developed. The obtained lysate was tested on growth of 15 NSLAB commonly found in cheese (13 commercially available and two isolated from vintage Cheddar cheeses bought at local market in Copenhagen) and two SLAB representatives (from undefined starter culture). As shown in figures 3a+b for two strains (*Lactobacillus rhamnous and Lactobacillus delbrueckii*), NSLAB strains showed significantly increased growth potential when lysed SLAB cells were added to the media. In total 13 of the 15 NSLAB strains tested showed increased growth potential, while the two SLAB representatives (also present in the digested starter culture) did not show any increased growth potential. Preliminary results also indicate that for some of the NSLAB strains, the lag phase might also to some extent be affected by the addition of SLAB lysate to the media.

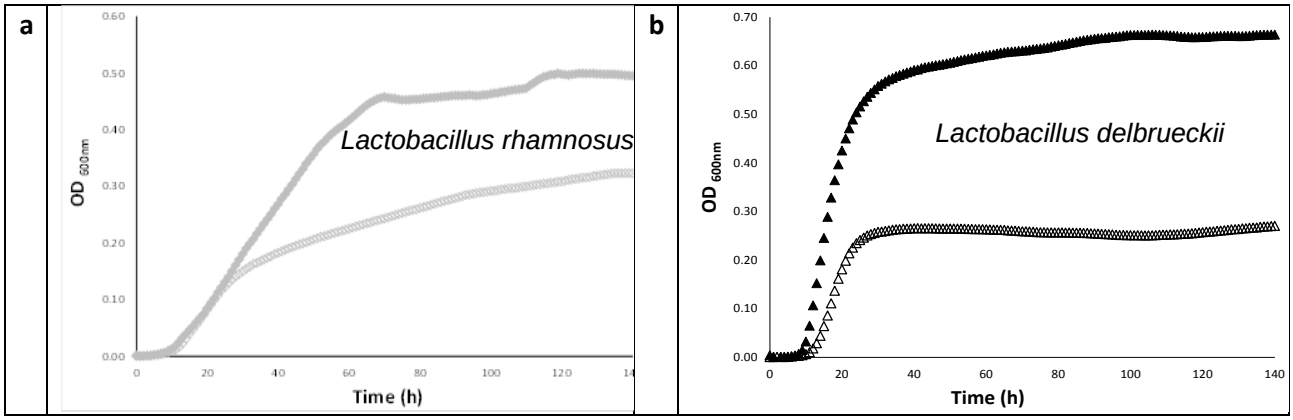

Figure 3: Optical Density ($OD_{600\ nm}$) measured during growth of NSLAB at 30°C in LM17 broth (deficient of lactose), supplemented (closed symbols), or not (open symbols), with 10 % lysate from enzymatic digested SLAB.

Work package 3

A method based on the principle of Fluorescent Recovery after photobleacing (FRAP) has been established to determine diffusion of different metabolites in cheese.

Diffusion measurements have been carried out on peptides originating from casein with sizes of 10 AA (amino acids), 23 AA and 50 AA. The reason for choosing peptides was that amino acids are an important source for NSLAB to ensure optimal ripening of the cheese. Both positively, negatively charged and neutral variants of the peptides were tested.

An analytical model for determination of diffusion rates was developed based on the Soumpasis model, and applied to fit the data sets produced with the confocal microscope using the FRAP software package associated with the microscope. Figure 3 shows an example of model fitting data from a diffusion experiment in the Cheddar cheese. For each peptide, at least 20 of such FRAP datasets were made and fitted using the Soumpasis model.

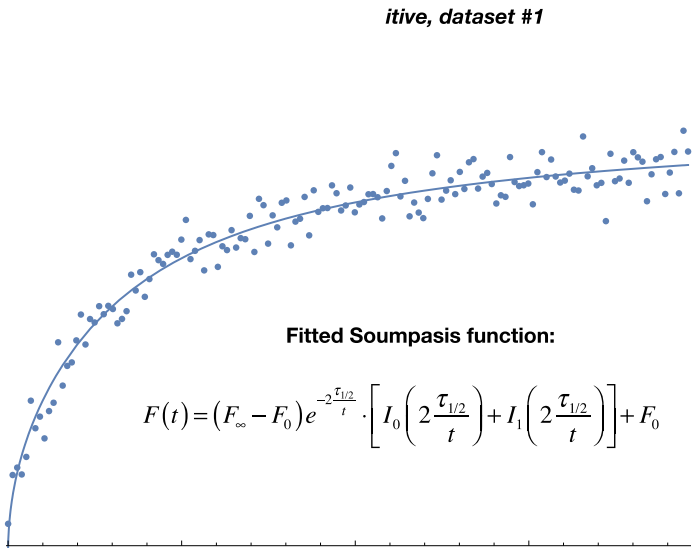

Figure 3: Example of applying the Soumpasis model fitted to measured FRAP data.

For the 23 AA only the positively charged peptide was tested, because we encountered problems with the solubility of the two other 23 AA peptides when applying them to the cheese.

In table 2 below, the average diffusion rates are provided. Compared to previous studies carried out in slightly less complex cheese model systems (Casein/milk concentrate gel system) (Silva et al., 2015). The diffusion rates of the peptides (ranging in size from ca. 1 KDa-5 KDa) in a real Cheddar cheese ranges from the same order of magnitude as the largest molecules (2000 KDa) tested in the milk concentrate model to nearly ten times lower. This clearly indicates that the diffusion in a real cheese system is significantly lower than previous studies in model systems have indicated. However, despite these significantly lower diffusion rates, our modelling studies in WP1 still indicates that diffusion is not a limiting factor for ripening of Cheddar cheese.

Table 2. Average diffusion rates measured for different peptides.

Peptide	Diffusion rate (D_W) (mm^2/hour)
10 AA-neutral	0,00393
10 AA-positive	0,00664
10 AA-negative	0,00986
23 AA positive	0,00147
50 AA-neutral	0,00146
50 AA-positive	0,00079
50 AA-negative	0,00327

Apart from showing generally much lower diffusion rates of peptides as originally expected based on our initial observations from literature, the results in terms of size of peptide and peptide charge are still rather inconclusive. The results are indeed interesting, but would need to be repeated before publication. This will require new fluorescently labelled peptides, which is rather expensive, as well as thorough repetition of all experiments.

Conclusion:

The overall conclusion is that by applying mathematical modelling, completely new light has been shed on the cheese ripening process. Diffusion is most probably not an in important factor in the ripening process. A method to evaluate the diffusion rate has been developed and implemented. This model will allow for the investigation of the diffusion of many different metabolites, at many different conditions. In reality, it is probably only rather large molecules that is prevented from diffusing freely and at rather high rate in the cheese matrix. However, it is most doubtful if such large molecules ever become limiting for any ripening steps in the cheese. Our modelling data has led to a slight change of plans in WP 2, moving all our resources into investigating how to boost the growth of NSLAB using different sugars as an additional energy source. Unfortunately, the results from the more than one year long experiment were inconclusive, because the NSLAB never established in the Cheddar cheese. On the other hand, these studies may shed light on how SLAB contributes to the cheese ripening in the absence of NSLAB development.

11. Deviations

9.1 Scientific

With the slight change in project plan priorities as also explained above and in previous reports, the project has finalized within time.

11.2 Financial

All delays in timetable as indicated below has been carried out within the financial frame of the project.

11.3 Timetable

Work package 1
Establishment of flavor formation model is not possible with existing literature. It is also not realistic to setup experiments to support such generation of quantitative data on aroma formation.

Work package 2
The time it takes to make cheese trials is very long (+6 month per experiment). It was not possible within the timeframe of the project to carry out a new experiment where we could add NSLAB.

Work package 3
The developed FRAP method to study metabolite diffusion is expensive and time consuming. It was not possible within the existing timeframe (and economy) to carry out studies at different physiological conditions.
Development of alternative methods was not necessary, because the existing method works very well.

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

Scientific relevance

- A new concept has been established in cheese ripening, where mathematical modeling and experimental trials are combined to better understand the cheese ripening process.
- A model has been developed to predict how best to optimize the cheese ripening process.
- A whole new understanding of the importance of diffusion in the cheese ripening process has been achieved.
- New knowledge has been established to document the development of starter culture and the development of taste, etc., when non-starter bacteria are not established during cheese ripening.
- A method has been developed and used to study the diffusion of peptides directly in cheese. The diffusion seems to be significantly slower than observed in previous studies (from literature), where diffusion was studied in slightly more well-defined cheese-like structures.

Relevance for the society

- As we start to understand the diffusion mechanisms of metabolites and growth of microorganisms, we will also become better at optimizing the conditions to accelerate ripening. Accelerated ripening means shorter time to market and shorter time at storage, which reduces energy used for cooling, etc.

Relevance for the industry

- It will take time before the mathematical modelling will be implemented in industry, but the understanding of diffusion provided in this project is highly relevant for industry, and may help guide industry towards improved ripening processes in the future.

Future projects

Project I – Boosting NSLAB growth during ripening

Having ruled out that diffusion is not a limiting step for diffusion, we can start to look for other parameters of relevance to accelerate ripening. Unfortunately, the studies carried out in this project did not give any conclusive results on how to boost the growth of NSLAB during ripening. However, this should be the next place to look. A defined set of experiments should be developed to determine, which parameters are of relevance to enhance NSLAB growth. First, we need to determine if the slow establishment of NSLAB is due to a long lag phase of NSLAB and rather lack of the right nutrients for growth. Next, we need to understand which nutrients can supply NSLAB growth without compromising taste or other relevant cheese quality parameters. Finally, we need to supply protocols that enable the cheese makers to implement a possible boost of NSLAB growth in the initial phase of cheese ripening. This may be relatively easy to implement during the salting phase of Cheddar cheese production, but for other cheeses implementation could be more complicated.

Project II – Flavor formation model

Our literature studies have shown a suppressing lack of structured studies to understand how flavor develop over time. Mathematical modelling combined with the “right” flavor formation studies can help structuring this process. Detailed studies of the flavor formation process should be carried out on selected cheeses and models for the most relevant flavor developments should be established. A flavor model will probably never be able to exactly model the flavor formation process, but again, combining this model with that of NSLAB growth we will acquire valuable insight into how NSLAB growth and flavor formation modelling can be combined to guide the right and most optimal conditions for accelerated ripening without compromising quality.

13. Communication and knowledge sharing about the project

The project has been presented in Mælkeritidende and on FOODs home page (http://food.ku.dk/nyheder/ostemodningsmatematik/)

Presentations at conferences:

- Møller C.O.A., Czárán T., Siegumfeldt H., Christensen B.B., Rattray F.P. (2016) Reduction of Cheddar cheese ripening time through the addition of glucose. In: Final Programme and Abstract Book, 7th Danish Microbiological Society Annual Congress 2016, 14th November 2016, Copenhagen, Denmark.

- Czárán T., Rattray F.P., Møller C.O.A., Christensen B.B. (in preparation) Modeling the load of metabolite diffusivity on growth rate of non-starter lactic acid bacteria during cheese ripening. 10th International Conference on Predictive Modelling in Food, Córdoba, Spain.

Papers with peer review:

- Czárán T., Rattray F.P., Møller C.O.A., Christensen B.B. Modelling the influence of metabolite diffusion on non-starter lactic acid bacteira growth in ripening Cheddar Cheese. International Dairy Journal. 80: 35-45.

- Møller C.O.A., Czárán T., Siegumfeldt H., Christensen B.B., Rattray F.P. (in preparation) Cheddar cheese ripening and starter culture development in cheese deficient of NSLAB development. Short communication to be submitted to International Dairy Journal

- Møller C.O.A., Czárán T., Siegumfeldt H., Christensen B.B., Rattray F.P. (in preparation) Analyzis of peptide diffusion (different sizes and charges) in Cheddar cheese. To be submitted to a Food and/or Dairy Journal.

- Møller C.O.A., Christensen B.B., Rattray F.P. (in preparation) Investigation of NSLAB growth on lysed SLAB cells. To be submitted to Food or Dairy Journal.

14. Contribution to master and PhD education

A two months project was developed as part of a master student study. The activities developed were related to isolating and identified NSLAB (by 16S gene sequencing) from commercial vintage Cheddar cheeses. Setting up of a enzymatic method for N-acetyl glucosamine determination was also initiated by the student.

15. New contacts/projects

The project has established collaboration between the initial partner involving representatives from Arla, Niels Bohr institute and KU Food. This collaboration might become very valuable for future projects involving mathematical modelling of cheese development.

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.

Signature, Project manager: Bjarke Bak Christensen, 14 May 2018

Signature, DDRF-representative: Grith Mortensen, 14 May 2018