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BESPOKE – Skræddersyede syrnede mejeriprodukter; nye strategier til forståelse af og kontrol med interaktioner mellem mælke-proteiningredienser og mælkens øvrige bestanddele under procesbehandling

BESPOKE – Bespoke fermented dairy products; new strategies for understanding and controlling protein ingredient-milk component interactions during processing

Mejeribrugets
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Final report

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1. Title of the project

Danish: Skræddersyede syrnede mejeriprodukter; nye strategier til forståelse af og kontrol med interaktioner mellem mælkeproteiningrediенser og mælkens øvrige bestanddele under procesbehandling (BESPOKE)

English: Bespoke fermented dairy products; new strategies for understanding and controlling protein ingredient-milk component interactions during processing

2. Project manager

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Postdoc Wahyu Wijaya, DTU Bioengineering, Department of Biotechnology and Biomedicine (1 July 2019 – 30 November 2020)
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4. Sources of funding

DDRF (Mælkeafgiftsfonden)
Chinese Scholarship Council (CSC)
Arla Foods amba

5. Project period

Project period with DDRF funding:	September 2018 – February 2022
Revised, if necessary:	September 2018 – November 2022

6. Project summary

Danish:

Mælkeproteiningredienser anvendes i mejeriprodukter for at opnå proteinrige produkter med ønskede funktionaliteter. For syrnede produkter er struktur, tekstur og stabilitet afgørende egenskaber at forstå og kontrollere. Formålet med projektet var derfor at skabe en mere grundlæggende forståelse af samspillet mellem de proteiner, der naturligt findes i mælk, og tilsatte mælkeproteiningredienser, under forarbejdning, syrning og opbevaring. Derved skabes bedre muligheder for udvikling af produkter med skræddersyede egenskaber.

Det eksperimentelle arbejde i projektet blev udført i tre arbejdspakker. I arbejdspakke 1 blev der udviklet et modelsystem, der sikrede fleksibilitet og kontrol med proteinsammensætningen og god reproducerbarhed. Modelsystemet består af koncentrater af kaseiner og valleproteiner, produceret i pilotskala, opbevaret ved -80°C og optøet med en skånsom optøningsprocedure, samt tre forskellige valleproteiningredienser med potentiel anvendelse i syrnede mejeriprodukter: Valleproteinkoncentrat (WPC), mikropartikuleret valleprotein (MWP) og nanopartikuleret valleprotein (NWP). En række prøver blev formuleret, forarbejdet, syrnet og analyseret gennem hele produktionsprocessen og den efterfølgende opbevaring, hvilket førte til ny viden om forskelle mellem ingredienserne og demonstrerede modelsystemets anvendelighed.

I arbejdspakke 2 blev protein-protein interaktioner studeret mere detaljeret. Udgangspunktet var analyser af relativt enkle prøver af rene proteiner, for at opnå ny indsigt og udvikle metoder, der også kunne anvendes på prøver med højere kompleksitet. Der blev skabt ny forståelse af kaseiners selv-associering og interaktion med β-lactoglobulin samt af kaseiners chaperone-lignende aktivitet. Derudover blev interaktionerne mellem koncentraterne og ingredienserne fra arbejdspakke 1 analyseret, også under syrning.

I arbejdspakke 3 blev strukturen og stabiliteten af syrnede mælkegeler baseret på modelsystemerne studeret. Ikke-destruktive teknikker som Low Field Nuclear Magnetic Resonance og Small Angle X-ray Scattering blev anvendt til at nærmere kortlægge de strukturelle ændringer, der opstår under forarbejdning. Desuden blev der udviklet en ny metode til separat farvning af kaseiner og valleproteiner til højopløsningsmikroskopi hvilket gjorde det muligt at visuelt dokumentere nogle af de observerede forskelle mellem de anvendte ingredienser.

Projektet har således med succes udviklet et modelsystem og en række analytiske metoder til undersøgelse af interaktioner mellem tilsatte mælkeproteiningredienser og naturligt forekommende mælkeproteiner under forarbejdning og syrning. Derved er opnået helt ny specifik viden om de analyserede systemer, mens den analytiske platform også har vist stort potentiale til bredere anvendelse.

English:

Milk protein ingredients are used in dairy products to obtain protein rich products with desired functionalities. For acidified products, structure, texture and stability are pivotal properties to understand and control. Therefore, the aim of the project was to increase the fundamental understanding of interactions occurring between the proteins naturally present in milk and added milk protein ingredients during processing, acidification, and storage to enable tailoring and control of the final product properties.

The experimental work of the project was performed within three work packages. In work package 1, a model system was developed, allowing flexibility and control of protein composition, as well as good reproducibility. The model system was based on concentrates of caseins and whey proteins, produced in pilot scale, stored at -80°C and thawed using a gentle procedure, used together with three different whey protein ingredients with potential application in acidified dairy products: whey protein concentrate (WPC), microparticulated whey protein (MWP) and nanoparticulated whey protein (NWP). A range of samples were formulated, processed, acidified and analyzed throughout the production and storage, providing novel knowledge about differences between the ingredients and demonstrating the applicability of the model system.

In work package 2, protein-protein interactions were studied in greater detail. The outset was analysis of relatively simple samples of pure proteins, used to obtain increased insight and understanding and to develop a methodology that could also be used for samples with higher complexity. This facilitated novel understanding of the self-association of caseins and the interaction with β-lactoglobulin and the chaperone-like activity of caseins. Additionally, results were also obtained from studying the interactions between the concentrates and ingredients from work package 1, including analysis during acidification.

In work package 3, the structure and stability of acidified milk gels based on the model systems were studied. Non-destructive techniques like Low Field Nuclear Magnetic Resonance and Small Angle X-ray Scattering were applied to probe the structural changes occurring during processing. Furthermore, using a newly developed method for separate staining of caseins and whey proteins for high-resolution microscopy, it was possible to visualize some of the observed differences between the ingredients.

In conclusion, the project has successfully developed a model system setup and a range of analytical methods for investigation of interactions between added milk protein ingredients and naturally present milk proteins during processing and acidification. Thereby, new knowledge about the specific systems analyzed has been obtained, and the developed analytical platform holds major potential for broader application.

7. Project aim

Danish:
Det overordnede formål med projektet er at opbygge ny forskningsbaseret viden, der kan sikre en effektiv udvikling af nye syrnede mejeriprodukter med skræddersyet tekstur, optimeret stabilitet og specifikke ønskede funktionaliteter.

English:

The overall aim of the project is to provide a science platform to allow efficient development of new fermented dairy products with tailored texture, optimized stability and specific desired functionalities.

8. Background for the project

The project aims at gaining a new understanding of, and developing new strategies for control of the molecular interactions that occur between milk protein ingredients (MPI) and native milk proteins. This will be used to tailor the physical, chemical and functional characteristics of fermented dairy products (semisolid), during and after processing. To achieve this, complementary competences and infrastructure at three universities (KU SCIENCE, DTU and SDU) will be utilized. In addition, Arla Foods amba and Arla Foods ingredients will be involved.

The increasing global demand for protein rich dairy products and dairy products with low content of sugars and fat provides novel possibilities for the use of milk protein ingredients (MPIs). In order to enable the industry to tailor ingredients and develop new and sufficiently stable fermented products, a fundamental understanding of the chemistry governing interactions between milk constituents and MPIs and how this can be manipulated is necessary. This, in turn, will aid in developing the market potential for Danish MPIs and dairy products by providing a platform for development of new fermented products with a novel level of tailored texture, excellent stability, specific functionalities and robust, cost effective and energy efficient industrial scale production.

The main scientific objectives of the project are to:

- Fundamentally understand the type of binding involved when milk components and milk protein ingredients interact depending e.g. on degree of denaturation, ionic strength, molecular properites, pH and available calcium.
- Clarify how the molecular interactions affect the structure, texture and stability of fermented dairy products. Focus will be on how specific levels of free thiol groups in milk protein ingredients can be used to control the amount of protein interactions during heat treatment and subsequent acidification and how this affects the firmness and stability of fermented dairy products
- Optimize process parameters (e.g. heat and shear) in order to maximize desired functionality (physical stability, shininess, no graininess) of MPIs in fermented dairy products.
- Develop predictive models for stability and other macroscopic quality parameters

9. Sub-activities in the entire project period

Work package	Milestones	Status and comments
	Recruitment	PhD student starting September 2018, PhD thesis defended January 2022 Post doc 1 recruited from July 2019, resigned November 2020 Post doc 2 recruited from February 2021 – project end
WP1 Development of model systems	M 1.1 Model systems established M 1.2 Analytical methods developed and protocols established	Work package completed in 2020 2 scientific publications
WP2 Understanding protein-protein interactions	M.2.1 Effect of variations in processing and formulation on properties of model fermented products investigated M 2.2 Relation between molecular properties and macroscopic quality of fermented dairy products established	Work package completed in 2022 2 scientific publications + 2 manuscripts in preparation
WP3 Prediction of stability and other macroscopic properties	M 3.1 Methods for shininess and graininess established M 3.2 Range of fermented dairy products investigated for physical stability and other parameters during storage M 3.3 Fermented dairy products characterized using various imaging methods M 3.4 Data used to develop predictive models	Work package completed in 2022 with some changes Milestones 1-3 achieved with minor changes Milestone 4 only achieved to a very limited extent with some preliminary modelling efforts on data from WP2 and WP3 3 scientific publications
WP4 Verification	M 4.1 Pilot scale production established and products analysed M 4.2 Predictive model applied	No pilot scale productions were performed
WP5	Administration	Project meetings planned and held 3-4 per year, alternating between online and physical meetings hosted by the project partners Final meeting in November 2022

10. Deviations

Scientific: The expected results within predictive modelling and pilot scale process verification were not obtained, partly due to a slight change of focus in WP2 and WP3 with a necessary increased effort in understanding the interactions between components at a molecular level and in characterization of properties and microstructure of the gelled model systems. Partly this was also inflicted by the limitations in mobility and experimental delays caused by the corona lockdowns.

Time and economy: The project was extended from February 2022 to November 2022 without need for additional funds, because of late start of post doc 1, time gap between post doc 1 and 2 as well as lower than budgeted salary for post doc 1. An extension of the PhD study for 3 months due to corona-virus related delays were given as well.

Due to the retirement of Professor Richard Ipsen in 2020, the project management and role as main supervisor of the PhD student was handed over to Associate professor Anni Bygvrå Hougaard. Richard Ipsen continued active participation in the project throughout the project period as Professor emeritus.

11. Project results

Work package 1 (WP1):

The first task of the project was establishment of a model system, which could provide flexibility in terms of protein composition and ensure reproducibility between studies. Hence, concentrates of caseins and whey proteins were produced by membrane filtration of skim milk in pilot scale, using microfiltration (MF) to separate and concentrate the caseins, followed by ultrafiltration of the permeate to collect the whey proteins. The UF permeate was collected and used as dilution medium later. Both concentrates were frozen at –80°C for storage, whereas the permeate was stored at –20°C. The thawing procedure was found to significantly affect the properties of the thawed concentrates, and it was found that it was possible to obtain properties, including acid gelation, similar to unfrozen concentrates when applying a gentle thawing procedure with storage for 3 days at 4°C (in 15 ml tubes) followed by 30°C for 30 minutes, and using permeate for dilution to adequate protein concentration. This thawing procedure was used for all subsequent studies.

The results regarding thawing of frozen concentrates are published in: Li, R., Rovers, T.A.M., Jæger, T.C., Hougaard, A.B., Svensson, B., Simonsen, A.C., Ipsen, R. (2021). Effect of thawing procedures on the properties of frozen and subsequently thawed casein concentrate. *International Dairy Journal*, *112, 104860*. https://doi.org/10.1016/j.idairyj.2020.104860

The concentrates were then used together with three different whey protein ingredients with potential application in acidified dairy products (whey protein concentrate (WPC), microparticulated whey protein (MWP) and nanoparticulated whey protein (NWP), all available in powder form). Acidification was obtained by addition of glucono-δ-lactone (GDL). Model systems were designed to have equal concentrations of casein and whey protein, and with the whey protein fraction consisting of the liquid whey protein concentration produced for the project in combination with one or two of the whey protein ingredients reconstituted from powder. The properties of the model systems were characterized throughout a simulated process, i.e. after homogenization, pasteurization, acidification and storage (7 days, 5°C) on a range of properties, including surface hydrophobicity, surface thiols, particle size, gelation time and pH, rheological properties of the gel, water holding capacity and graininess. The results showed marked differences between the model systems, especially for the particulated ingredients, MWP and NWP, which were attributed to different behavior of the added whey protein ingredients when interacting with the milk constituents during processing. Addition of NWP led to rapid gelation and formation of a stiffer gel compared to MWP, however, NWP was also correlated to higher level of graininess. Systems with mixing of the two ingredients seemed to be dominated by the properties observed when adding NWP, though a lower level of graininess was observed. Thereby it was also confirmed that the model systems and the selected analyses were useful in characterization and, to some extent, quantification of differences in ingredient behavior under simulated application conditions.

These results are published in: Li, R., Rovers, T.A.M., Jæger, T.C., Wijaya, W., Hougaard, A.B., Simonsen, A.C., Svensson, B., Ipsen, R. (2021). Interaction between added whey protein ingredients and native milk components in non-fat acidified model systems. *International Dairy Journal*, *115, 104946*. https://doi.org/10.1016/j.idairyj.2020.104946

Work package 2 (WP2):

This work package was aimed at increasing the understanding of the protein-protein interactions occurring in acidified milk systems containing natural milk proteins as well as whey protein ingredients. The fulfillment of this aim required development of methodology able to handle the high level of complexity of such systems. The work was therefore initiated by studies of the behavior of simple samples consisting of pure proteins in relatively dilute buffer solutions to characterize and understand the interactions leading to self-association and micelle formation. Further studies included interactions occurring during heating, and the effects of the presence and concentration of calcium. This was then later progressed towards higher complexity by studies including the protein concentrates produced based on WP1 and the whey protein ingredients (WPC; MWP and NWP).

The initial study led to the conclusion that the physical properties of β-/κ-casein mixed micelles can be tuned by interactions between these two different intrinsically disordered proteins. The molar ratio between β- and κ-caseins plays a key role for the micellar characteristics, assigning specific contributions to β- and κ-casein in controlling size, occupying the interior and decorating the exterior of the micelles. The interactions were studied using isothermal titration calorimetry, dynamic light scattering, fluorescence measurements and small-angle X-ray scattering.
Published in: Wahyu Wijaya, Sanaullah Khan, Mikkel Madsen, Marie Sofie Møller, Tijs Albert Maria Rovers, Tanja Christine Jæger, Richard Ipsen, Peter Westh, Birte Svensson (2021): Tuneable mixed micellization of β-casein in the presence of κ-casein. *Food Hydrocolloids, 113, 106459.* https://doi.org/10.1016/j.foodhyd.2020.106459

In the following study, interactions between the individual caseins (α-, β-, and κ-casein) and β-lactoglobulin (β-Lg) were studied, during heating and in the presence of different concentrations of calcium. This included introduction of new methods allowing more dynamic measurement of changes occurring during heating and cooling, though still in simple dilute samples. The results here showed that that the caseins and β-Lg behaved differently at different temperatures and calcium concentrations. While heat treatment affected the denaturation and aggregation of β-Lg with little dependency on calcium, α-casein and β-casein were influenced considerably by calcium. Chaperone-like activity of caseins for β-Lg has been described previously by others, and here we concluded that caseins display chaperone activity through slight suppression of β-Lg unfolding and that this process is also dependent on the type of caseins. Furthermore, the chaperone activity of caseins significantly limits the aggregate formation of unfolded β-Lg in the absence of calcium or decreases the aggregate size in the presence of calcium.
This is illustrated in figure 1, and described in the publication (where the figure is also inserted from): Hossein Mohammad-Beigi, Wahyu Wijaya, Mikkel Madsen, Yuya Hayashi, Ruifen Li, Tijs Albert Maria Rovers, Tanja Christine Jæger, Alexander K. Buell, Anni Bygvrå Hougaard, Jacob J.K. Kirkensgaard, Peter Westh, Richard Ipsen, Birte Svensson (2023): Association of caseins with β-lactoglobulin influenced by temperature and calcium ions: A multi-parameter analysis. *Food Hydrocolloids, 137, 108373.* https://doi.org/10.1016/j.foodhyd.2022.108373

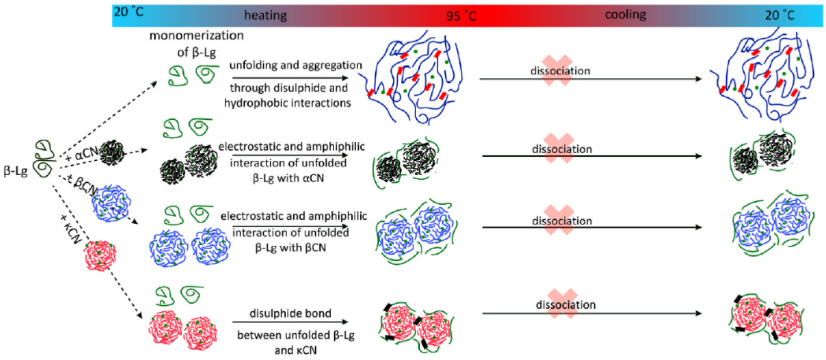

Fig. 7. Chaperone-like effects of CNs to reduce the aggregation of β-Lg. CNs reduce the aggregation of β-Lg through interaction with unfolded β-Lg. The aggregates formed between β-Lg and the CNs at high temperature are stable and do not dissociate upon cooling.

Figure 1: Illustration of chaperone like activity of caseins for β-lactoglobulin during heating and cooling. From: Hossein Mohammad-Beigi, Wahyu Wijaya, Mikkel Madsen, Yuya Hayashi, Ruifen Li, Tijs Albert Maria Rovers, Tanja Christine Jæger, Alexander K. Buell, Anni Bygvrå Hougaard, Jacob J.K. Kirkensgaard, Peter Westh, Richard Ipsen, Birte Svensson (2023): Association of caseins with β-lactoglobulin influenced by temperature and calcium ions: A multi-parameter analysis. *Food Hydrocolloids, 137, 108373.* https://doi.org/10.1016/j.foodhyd.2022.108373

The chaperone-like activity of caseins was also observed in samples containing the whey protein ingredients, thus increasing the stability of the particles at high temperature by preventing the aggregation otherwise seen (unpublished results).

Within work package 2, results have also been obtained regarding self-association of the individual caseins into micelle structures and the influence of calcium on stability. We have developed an advanced analytical package that combines Dynamic Light Scattering (DLS) and Taylor Dispersion Analysis (TDA) as a pioneering platform for size-based characterization and stability analysis of protein aggregates in microcapillaries. Additionally, Mass Photometry was incorporated to detect low-order casein aggregates, which are often undetectable with other conventional techniques. The results are not published yet, and therefore, not described in further detail here.

Similarly, results have also been produced studying the interactions between the protein concentrates produced based on WP1 and the whey protein ingredients (WPC; MWP and NWP), i.e. in samples of higher complexity than the initial studies of the work package, and even studies of interactions occurring during acidification and gel formation were performed, including visualization of the gel structures with cryo-SEM. These results are also not described in further detail here, because they are not published yet.

Work package 3 (WP3):

The final aim of WP3 was to develop predictive models for the stability and other macroscopic properties of acidified milk gels. The modelling was not achieved, but a range of advanced methods for characterization of the acid gels were developed. These are based on, among others, high-resolution microscopy (Stimulated Emission Depletion (STED)), low-field nuclear magnetic resonance (LF-NMR) spectroscopy and small-angle X-ray scattering (SAXS).

LF-NMR was applied to study the water mobility in acidifying model systems and during storage of the acidified milk model gels. During acidification, two populations of protons (water) were identified, and the actual relaxation time was slightly dependent on the sample composition, and the abundance of each population shifted, showing that water in the acidifying systems was shifting from being less mobile towards higher mobility. Differences in the rate of shift were observed between the added whey protein ingredients. The acidified milk model systems were subjected to a simulated stirring/smoothing process, stored for 14 days at 5°C, and analyzed several times during the storage period. After the storage period, LF-NMR detected three populations of water, with a new fraction identified as free water, and interpreted as spontaneous syneresis. The LF-NMR results were combined with results from physical stability analyses as well as quantitative image analysis of confocal laser scanning microscopy (CLSM) images and correlations

between the results analyzed, showing a clear correlation between water mobility and microstructure of the acidified gels. As LF-NMR is a non-destructive method and good correlations to microstructure and stability were seen as well as differences between the added protein ingredients, the method shows potential in future research efforts aimed at prediction of stability.
The results are published in: Li, Ruifen, Czaja, Tomasz Pawel, Glover, Zachary J., Ipsen, Richard, Jæger, Tanja Christine, Rovers, Tijs Albert Maria, Simonsen, Adam Cohen, Svensson, Birte, van der Berg, Franciscus Winfried J & Hougaard, Anni Bygvrå (2022). Water mobility and microstructure of acidified milk model gels with added whey protein ingredients. *Food Hydrocolloids*, *127, 107548*. https://doi.org/10.1016/j.foodhyd.2022.107548.

SAXS, another nondestructive method with potential for analysis of structural changes in opaque samples, was applied to detect changes in caseins and whey proteins during heating and acidification, showing that the caseins were largely unaffected by heat treatment, whereas structural changes were observed in the whey proteins. During acidification the opposite was observed, as the caseins were more heavily affected than the whey proteins. In mixed systems of caseins and the different whey protein fractions, the overall behavior could be understood as a combination of the individual effects, and the type of added whey protein components influenced the resulting structure formation and dynamics. Further structural analysis and visualization was obtained by development of a separate staining procedure for casein and whey proteins followed by processing by heating and acidification before analysis by STED microscopy. Figure 2 shows images of acidified gels containing caseins together with each of the three whey protein ingredients studied during the project, with the caseins appearing in green and the whey proteins in red. The images clearly show differences between the positioning and interactions between the caseins and the whey protein ingredients.

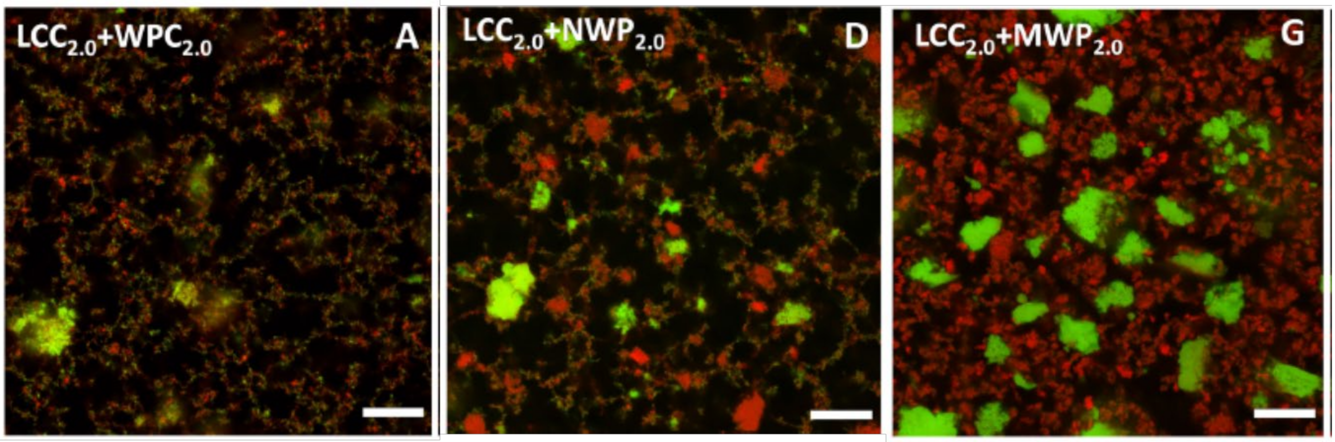

Figure 2: STED images of acidified gels of milk model systems. LCC: Liquid casein concentrate. WPC: Whey protein concentrate. NWP: Nanoparticulated whey protein. MWP: Microparticulated whey protein. Each protein is present in 2.0% concentration (w/w). Casein appear in green and whey proteins in red in the images. Excerpt from figure 5 in: Li, R., Ebbesen, M.F., Glover, Z.J., Jæger, T. C., Rovers, T. A. M., Svensson, B., Brewer, J.R., Simonsen, A.C., Ipsen, R., & Hougaard, A.B. (2023): Discriminating between different proteins in the microstructure of acidified milk gels by super-resolution microscopy. *Food Hydrocolloids*, *138, 108468*. https://doi.org/10.1016/j.foodhyd.2023.108468

These results are published in the papers: Li, Ruifen, Jæger, Tanja Christine, Rovers, Tijs Albert Maria, Svensson, Birte, Ipsen, Richard, Kirkensgaard, Jacob Judas Kain & Hougaard, Anni Bygvrå (2022). In situ SAXS study of non-fat milk model systems during heat treatment and acidification. *Food Research International*, *157, 111292*. https://doi.org/10.1016/j.foodres.2022.111292 and Li, Ruifen, Ebbesen, Morten F., Glover, Zachary J., Jæger, Tanja Christine, Rovers, Tijs Albert Maria, Svensson, Birte, Brewer, Jonathan R., Simonsen, Adam Cohen, Ipsen, Richard, & Hougaard, Anni Bygvrå (2023): Discriminating between different proteins in the microstructure of acidified milk gels by super-resolution microscopy. *Food Hydrocolloids*, *138, 108468*. https://doi.org/10.1016/j.foodhyd.2023.108468

Taken together WP3 has resulted in development of new methodologies for characterization of acidified milk gels, and increased understanding of the interactions occurring, and differences observed, between gels produced with the different whey protein ingredients. A schematic suggestion of the formation of structure during processing is shown in figure 3, illustrating the non-interactive, somewhat destructive, effect of MWP, the self-aggregation of NWP combined with interaction with caseins and whey proteins present in the milk/model system, and the interactions of WPC with caseins in a very similar way as other whey proteins present.

In addition to the published results, experiments were also performed using fluorescence spectroscopy for characterization of changes in protein structure during acidification, and tendencies were observed in changing levels of buried/exposed tryptophan during the acidification process. However, time did not allow for this to be further developed and interpreted.

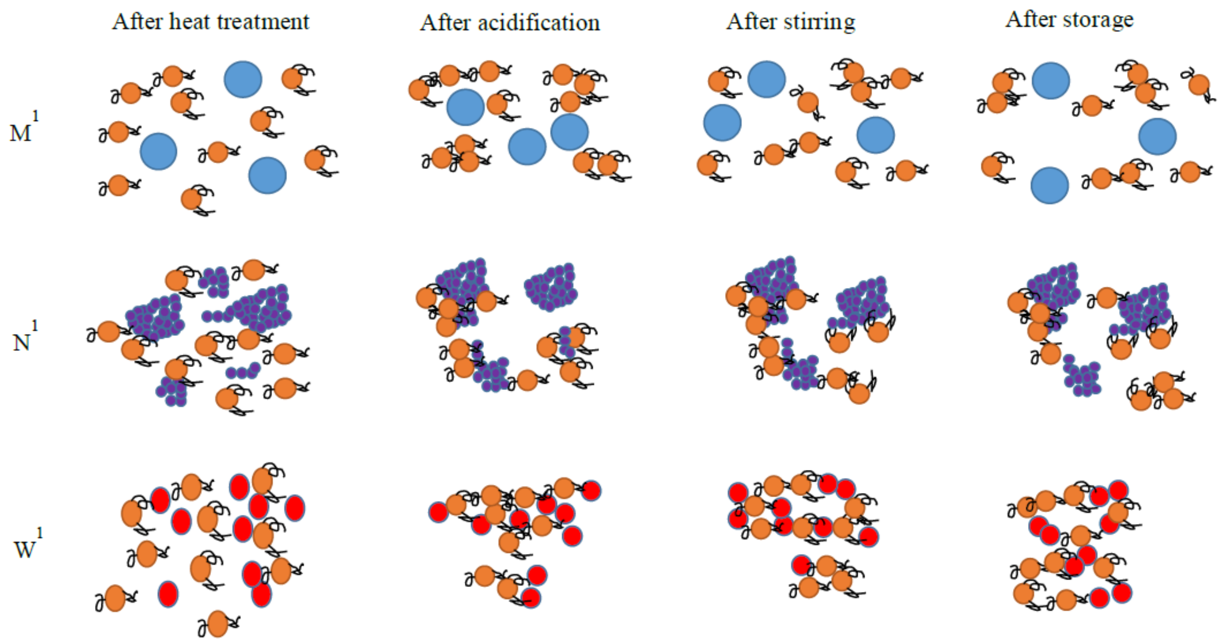

Figure 22. Diagrams of interacting inherent proteins with whey protein ingredients during processing of acidified gels. Inherent casein micelles (LCC) are represented by orange spheres, inherent whey protein (LWPC) are in black hairs, MWP are blue, NWP are purple, and WPC are red spheres. Not drawn to scale.

Figure 3: Suggested scheme of interactions occurring between caseins, whey proteins and added whey protein ingredients during the processing steps of acidified milk gel production. M: MWP: Microparticulated whey protein. N:NWP: Nanoparticulated whey protein. W:WPC:Whey protein concentrate. From: Ruifen Li, 2021, PhD Thesis.

Conclusion:

The project has successfully developed a model system setup and a range of analytical methods for investigation of interactions between added milk protein ingredients and naturally present milk proteins during processing and acidification, including analyses of fundamental protein-protein interactions in samples of higher complexity and under more dynamic conditions than previously seen. The project has thereby increased the understanding of interactions occurring between different protein fractions and the behavior of the added protein ingredients. The intended modelling and verification in pilot scale was not achieved, primarily due to time constraints and prioritization of already initiated work. Furthermore, the platform of analytical techniques could be highly valuable in studies of protein-protein interactions in other (similar) matrices.

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

The results have provided knowledge for input to the development of tailored texture and specific functionalities of dairy products (in industrial scale production) using tailor-made whey protein ingredients and increased the understanding of the behavior of the specific ingredients applied in the project. Furthermore, even though the intended predictive modelling of stability of acidified milk gels was not achieved, the model system setup and the methods developed may serve as basis for exactly that in a continued line of work.

The results of the project also provided an analytical platform spanning from fundamental to application-based analyses across different scales – here applied for analysis of interactions between milk proteins (as they are found in milk) and added milk protein ingredients in acidified milk gels. This platform could be expanded, extended and optimized for further use in both similar systems and even for inclusion of other types of ingredients and product types.

13. Communication and knowledge sharing about the project

Scientific papers in peer-reviewed journals:

1. Li, R., Rovers, T.A.M., Jæger, T.C., Hougaard, A.B., Svensson, B., Simonsen, A.C., Ipsen, R. (2021). Effect of thawing procedures on the properties of frozen and subsequently thawed casein concentrate. *International Dairy Journal*, *112, 104860*. https://doi.org/10.1016/j.idairyj.2020.104860
2. Li, R., Rovers, T.A.M., Jæger, T.C., Wijaya, W., Hougaard, A.B., Simonsen, A.C., Svensson, B., Ipsen, R. (2021). Interaction between added whey protein ingredients and native milk components in non-fat acidified model systems. *International Dairy Journal*, *115, 104946*. https://doi.org/10.1016/j.idairyj.2020.104946
3. Li, R., Czaja, Tomasz Pawel, Glover, Z. J., Ipsen, Richard, Jæger, T. C., Rovers, T. A. M., Simonsen, A. C., Svensson, B., van der Berg, Franciscus Winfried J & Hougaard, Anni Bygvrå (2022). Water mobility and microstructure of acidified milk model gels with added whey protein ingredients. *Food Hydrocolloids*, *127, 107548*. https://doi.org/10.1016/j.foodhyd.2022.107548
4. Li, R., Jæger, T. C., Rovers, T. A. M., Svensson, B., Ipsen, Richard, Kirkensgaard, Jacob Judas Kain & Hougaard, Anni Bygvrå (2022). In situ SAXS study of non-fat milk model systems during heat treatment and acidification. *Food Research International*, *157, 111292*. https://doi.org/10.1016/j.foodres.2022.111292
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6. Wahyu Wijaya, Sanaullah Khan, Mikkel Madsen, Marie Sofie Møller, Tijs Albert Maria Rovers, Tanja Christine Jæger, Richard Ipsen, Peter Westh, Birte Svensson (2021): Tunable mixed micellization of β-casein in the presence of κ-casein. *Food Hydrocolloids, 113, 106459.* https://doi.org/10.1016/j.foodhyd.2020.106459
7. Hossein Mohammad-Beigi, Wahyu Wijaya, Mikkel Madsen, Yuya Hayashi, Ruifen Li, Tijs Albert Maria Rovers, Tanja Christine Jæger, Alexander K. Buell, Anni Bygvrå Hougaard, Jacob J.K. Kirkensgaard, Peter Westh, Richard Ipsen, Birte Svensson (2023): Association of caseins with β-lactoglobulin influenced by temperature and calcium ions: A multi-parameter analysis. *Food Hydrocolloids, 137, 108373.* https://doi.org/10.1016/j.foodhyd.2022.108373

Manuscripts in preparation (first author Hossein Mohammad-Beigi):

Taylor Dispersion Analysis of Micellization (TDAM): Unveiling α, β, and κ caseins self-assembly (tentative)

The interaction of whey protein ingredients with native milk proteins changes the microstructure of yogurt gels *(tentative)*

Easily read papers: 1 (+ 1 upcoming)

Richard Ipsen, Ruifen Li, Birte Svensson, Ulf Andersen, Tanja Jæger 2019: Skræddersyede syrnede Mejeriprodukter, Mælkeritidende Nr. 13

Student theses: 2

Rasmus Søgaard Hansen, MSc thesis, Food Science and Technology, University of Copenhagen, 2019: Elucidation of differences between non-frozen and frozen micellar Casein Concentrates

Camilla Jørgensen, MSc thesis, Food Science and Technology, University of Copenhagen, 2021: Effect of added whey protein ingredients on selected properties of fermented non-fat milk model systems based on native milk components

Oral presentations at scientific conferences, symposiums etc.: 3

Ruifen Li, Richard Ipsen, 2019: Properties of fresh milk protein ingredients as a consequence of frozen storage, 8th International Symposium on Food Rheology and Structure - ISFRS 2019, June 17 - 20, 2019, Zürich

Ruifen Li, Anni Hougaard, Richard Ipsen, 2021: Image analysis for characterization of microstructure of non-fat acidified milk model systems with added whey protein ingredients. 4th Food Structure and Functionality Symposium, October 2021 (Online)

Anni Bygvrå Hougaard, Ruifen Li, Morten Frendø Ebbesen, Tanja Christine Jæger, Tijs Rovers, Zachary Glover, Jonathan Brewer, Adam Cohen Simonsen, Birte Svensson, Jacob Kirkensgaard, Richard Ipsen, 2023: Using SAXS and STED to elucidate how various whey protein ingredients function in acidified milk gels, 13th NIZO Dairy Conference, 17-20 October 2023, Papendal, The Netherlands

Oral presentations at meetings: 1

Hossein Mohammad-Beigi: "Use of FIDA to determine the factors influencing micelle formation in milk", First global Fidabio User Group Meeting, Copenhagen, 5-6 October 2022

Presentations at DDRF meetings during the project period

Other:

Ruifen Li, 2021: Understanding and controlling protein ingredient-milk component interactions during processing, PhD Thesis, University of Copenhagen

14. Contribution to master and PhD education

PhD: Ruifen Li, UCPH

MSc in Food Science and Technology, UCPH: Rasmus Søgaard Hansen and Camilla Jørgensen

15. New contacts/projects

The initial project group was expanded by connecting to the Biophysics platform at DTU and Professor Alexander Buell, and the project has provided a further strengthening of the connection between the project partners.

The developed scientific platform was suggested applied and further developed in a new project: Understanding milk and plant protein interactions (MILPRO) by application for funding by DDRF 2023, which was rejected and has so far not been resubmitted.