SLUTRAPPORT

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Forståelse af hvorledes mælkeproteiningredienser reagerer med andre mælkekomponenter som konsekvens af procesbehandling og hvordan dette påvirker fremstillede produkters slutkvalitet (PROCOMP)

Mejeribrugets
ForskningsFond

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Final report

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

1. Title of the project

Understanding how added milk protein ingredients interact with other milk components during processing and how this affects final dairy product quality (PROCOMP)

Forståelse af hvorledes mælkeproteiningredienser reagerer med andre mælkekomponenter som konsekvens af proces-behandling og hvordan dette påvirker fremstillede produkters slutkvalitet (PROCOMP)

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4. Sources of funding

Danish Dairy Research Foundation: 1,593,000 DKK

Chinese Scholarship Council (PhD student): 1,500,000 DKK

Arla Foods (self-financing): 400,000 DKK

KU and DTU (self-financing): 411,438 DKK

5. Project period

Project period with DDRF funding:	16/9-2013 to 15/9 2016
Revised, if necessary:	16/9-2013 to 31/7-2018

6. Project summary

Aim

The overall aim was to understand and quantify how various added milk protein ingredients interact during processing with casein micelles and fat globules in the milk and how this affect the final quality of fermented dairy products.

Main results

The project has generated important novel knowledge for application of whey protein-derived ingredients in fermented dairy products. It has been elucidated how the characteristics of whey protein aggregates, especially the amount of thiol groups and the surface hydrophobicity, affect interaction with other milk components. The project has also cast additional light on how these interactions determine performance in the final applications, with focus on fermented dairy products. The importance of hydrophobic and other non-covalent interactions for whey protein ingredient performance in dairy systems, previously somewhat neglected, has become evident.

In addition, the project has illustrated both advantages and limitations of using milk model systems and the obtained results will aid in the design of future systems used in more fundamental interaction studies. PROCOMP has also shown the applicability of using Isothermal Titration Calorimetry for obtaining detailed information on the interaction of the various components with e.g. calcium in very complex and heterogeneous systems such as commercial whey protein ingredients.

Conclusion

The obtained results and insights provide new possibilities for design and development of whey protein aggregates as ingredients in dairy products. The experience obtained with model milk systems and analytical techniques shows promise for future work and could possibly be extended to include protein-protein interactions as was the original intention.

Mål

Det overordnede mål var at forstå og kvantificere, hvordan forskellige tilsatte mælkeproteiningredienser reagerer under procesbehandling med kaseinmiceller og fedtkugler i mælken, og hvordan dette påvirker slutkvaliteten af fermenterede mejeriprodukter.

Hovedresultater

Projektet har genereret væsentlig og ny viden om anvendelse af valleprotein-ingredienser i fermenterede mejeriprodukter. Det er blevet belyst, hvordan egenskaberne af partikulerede valleproteinprodukter, især deres indhold af frie thiol-grupper samt overflade-hydrofobicitet, påvirker reaktionen med andre mælkekomponenter. Projektet har også belyst, hvordan disse interaktioner bestemmer slutkvaliteten af mejeriprodukter, med fokus på fermenterede produkter. Det er også blevet gjort helt tydeligt, hvor vigtige hydrofobe og andre ikke-kovalente interaktioner er for valleprotein-ingrediensers funktionalitet og virkemåde i mejerisystemer. Dette har ikke tidligere haft megen opmærksomhed.

Desuden har projektet illustreret både fordelene og begrænsningerne ved brug af modelsystemer for mælk, og de opnåede resultater vil medvirke til bedre at designe fremtidige systemer, der kan anvendes til mere grundlæggende studier af samspillet mellem ingredienser og mælkens bestanddele. PROCOMP har også vist anvendeligheden af isotermisk titrerings-kalorimetri til at opnå detaljeret information om interaktionen mellem forskellige komponenter i mælk og f.eks. calcium. Dette er illustreret i de meget komplekse og heterogene systemer, som kommercielle valleprotein ingredienser udgør.

Konklusion

Projektets resultater og den opnåede indsigt peger på nye muligheder for design og udvikling af produkter baseret på partikuleret valleprotein som ingredienser i mejeriprodukter. Erfaringerne opnået med modelsystemer for mælk samt med anvendte og analytiske teknikker kan med fordel anvendes i fremtidigt arbejde og kan muligvis udvides til at omfatte protein-protein-interaktioner, som det var den oprindelige hensigt i PROCOMP.

7. Project aim

The main objectives of the project are the following:

- To understand how aggregated protein ingredients with different surface reactivity interact during processing with casein micelles and fat globules in the milk.
- To quantify the magnitude and nature of these interactions.
- To clarify how the observed interactions affect final product quality, especially of fermented milk.
- To establish a knowledge base that will ensure a faster and more targeted development of future ingredients based on aggregation of milk proteins.

Projektets hovedmål er følgende:

- *At undersøge hvorledes aggregerede valleprotein ingredienser med forskellige overfladeegenskaber reagerer med mælkens kasein og fedtkugler ved fremstilling af mejeriprodukter.*
- *At kvantificere størrelsen og karakteren af disse interaktioner.*
- *At klarlægge hvorledes de undersøgte interaktioner påvirker slutkvaliteten af produktet, med særlig fokus på syrnede mejeriprodukter.*
- *At frembringe det teoretiske grundlag for en hurtigere og mere målrettet fremstilling af fremtidens vallebaserede ingredienser.*

8. Background for the project

Previous research conducted at University of Copenhagen (KU) in close collaboration with Arla Foods Ingredients clarified the importance of using the right milk protein aggregates as ingredients for fermented milk products. Microparticulated whey proteins (MWP) with different particle sizes and denaturation degree were investigated and compared to a standard full fat yoghurt. A high native to denatured whey protein ratio in the MWP powders provided yoghurts with high creaminess, viscosity, a slow meltdown in the mouth, creamy flavour, low syneresis and also an optimal microstructure of low fat yoghurt. Thus, whey protein aggregates added as ingredients should possess a high degree of reactivity with the protein in the milk during processing (i.e. heat treatment) to ensure a denser microstructure, less syneresis and a better sensory quality of fermented milks. It was also shown that both covalent and noncovalent interactions were involved in the formation of heat-induced complexes between whey protein, caseins and fat globules and that the surface reactivity.

Thus, to develop novel and optimized dairy ingredients based on processing of whey, it is of major importance not only to control the degree of aggregation of these ingredients, but also their surface properties and the resultant reactivity with casein micelles and fat globules during processing. The optimal reactivity is also product dependent, e.g. an ingredient suited for manufacture of low fat yoghurt would probably not be optimal in production of cheese or ice cream. The project PROCOMP was hence initiated to combine more fundamental model studies with applied research on dairy product quality in order to span the gap in our existing knowledge. The intention was to utilize a number of advanced techniques (surface plasmon resonance (SPR), isothermal titration calorimetry (ITC) and quartz crystal microbalance (QCM)) for characterizing and quantifying interactions between milk protein aggregates and inherent milk components (casein micelles, fat globules) in model systems designed to emulate milk. This would subsequently be related to properties of the aggregates (e.g. surface hydrophobicity, availability of sulfhydryl groups, content of native protein, hydration, particle size) as well as to final product quality (texture and sensory quality) and microstructure.

A range of whey protein ingredients (nano- and microparticulated whey protein products as well as whey protein isolate) with variable properties in relation to particle size, hydration, content of native whey protein etc. was made available for the project through Arla Foods Ingredients and could be applied in a range of milk model systems and subjected to processing equivalent to what takes place in industry. The achieved results could then subsequently be related to a rheological, microstructural and sensory characterization of pilot scale products made under similar processing conditions as the model systems.

The relevance of the project was to create a knowledge base for development of future natural, dairy-derived food ingredients and hence aid producers of protein-based dairy ingredients as well as producers of dairy products. Especially producers of fermented dairy products could benefit from being able to more intelligently choose dairy protein ingredients in order to ensure a high and consistent product quality. In addition, the project has the intention of developing novel methodologies for investigation and quantification of macromolecular interactions in complex foods, as well as for visualizing protein aggregate structure and hence deliver a novel, state-of-the-art technology platform of great value to future projects and collaborations between the participating partners.

In terms of scientific knowledge, the project was expected to generate novel, fundamental understanding of how macromolecular interactions determine structure formation and hence final product texture.

9. Sub-activities in the entire project period

WP 1: Development of methodology and model systems (9 months)

Model systems will be developed based on systems developed at KU. These systems have varying degrees of complexity ranging from a dispersion of milk protein aggregates in permeate from ultrafiltration (UF) to a simulated milk containing casein micelles, fat globules and native whey protein.

Selected milk protein ingredients will be investigated in these model systems and subjected to lab scale processing (heat treatment, homogenization, acidification/fermentation).

A strategy for further analysis will be developed, based on trials done with model systems using the planned instrumentation. Special attention will be given to SPR, ITC, QCM and confocal laser scanning microscopy (CLSM).

WP 2: Characterization of the behavior of selected milk protein ingredients in milk model systems as a consequence of processing into dairy products (9 months)

Selected aggregated milk protein ingredients will be characterized in terms of properties of the aggregates (e.g. surface hydrophobicity, content of native protein, available sulfhydryl groups, hydration (NMR), particle size) as a consequence of simulated processing (heat treatment, homogenization, acidification) in-to dairy products in model systems.

In addition, the texture will be investigated (rheology) and the microstructure characterized by confocal laser scanning microscopy using e.g. antibody staining for specific proteins to visualize how the protein ingredients are structured and how they are incorporated into the network of e.g. a fermented milk.

WP 3: Quantifying interactions between selected milk protein ingredients and casein micelles and fat globules (12 months)

Based on the results from WP 1 and 2, model systems and appropriate processing, conditions are chosen for further studies using ITC, QCM and SPR. For these selected systems, the interactions between milk protein aggregates and casein micelles and fat globules will be quantified and related to the performed model processing.

WP 4: Pilot scale trials to verify model studies (3 months)

The results obtained in WP 1-3 will be tested in pilot scale (i.e. 3-5 kg) to clarify the importance of the measured interactions for the final product quality. Fermented milk (yoghurt) will be the main focus. Thus, the same selected protein ingredients as in WP 3 will be used, and the products will be characterized in terms of textural and sensory properties.

WP 5: Writing of thesis (3 months)

Writing of final thesis.

Milestones, including final status:

	Month	Status
WP 1: Development of methodology and model systems		
1.1 Model systems developed	6	OK
1.2 A strategy for analysis developed	9	OK
WP 2: Characterization of the behaviour of selected milk protein ingredients in milk model systems as a consequence of processing into dairy products		
2.1 Selected aggregated milk protein ingredients characterized	15	OK
2.2 Method using confocal laser scanning microscopy and antibody staining for specific proteins developed	15	Decided to leave out
2.3 Microstructure of model fermented milks characterized by confocal laser scanning microscopy and rheology	18	OK
2.4 Scientific paper on characterization of the behaviour of selected milk protein ingredients in milk model systems as a consequence of processing into dairy products submitted	18	OK, paper published
WP 3: Quantifying interactions between selected milk protein ingredients and casein micelles and fat globules		
3.1 Model systems with appropriate processing developed	21	OK
3.2 Investigations using ITC performed	30	Completed January – March 2017. Scientific paper almost submission ready.
3.3 Investigations using SPR performed	30	Will not be undertaken
3.4 Investigations using QCM performed	30	Will not be undertaken
3.5 Scientific paper on quantification of interactions between selected milk protein ingredients and casein micelles and fat globules	33	OK (but without the fat globules). Two papers published
WP 4: Pilot scale trials to verify model studies		
4.1 Testing at pilot scale performed	33	OK
WP 5: Writing of thesis		
5.1 PhD thesis	36	Handed in 31st October 2016 and successfully defended 27th January 2017
5.2 Scientific paper on how interactions between selected milk protein ingredients with casein micelles and fat globules determine structure of fermented milks	36	OK and paper published

10. Project results

Results obtained in the PROCOMP project can be divided into the following areas:

1. Influence of added whey protein aggregates on rheological, textural and microstructural properties in acidified milk model systems.
2. Characteristics of whey protein ingredients (denaturation of whey protein and changes in whey protein ingredients during homogenization and heat treatment).
3. Interactions between whey protein aggregates and other proteins during processing in milk model systems (interactions occurring during homogenization, heat treatment and acidification).
4. Measurement of the interaction between whey protein ingredients and calcium using isothermal calorimetry.

Part 1:

Non-fat milk model systems were designed, based on dried milk ingredients (i.e microparticulated whey protein (MWP), nanoparticulated whey protein (NWP), whey protein isolate (WPI), micellar casein isolate (MCI) and ultrafiltrated whey permeate powder (UF). Experiments based on producing a number of systems with varying composition in order to elucidate what interactions occurred and how they influenced final product quality was set up. The systems were made at pilot plant scale (i.e. batches of 5 liters) and were subjected to homogenization (20 MPa), heat treatment (90 °C for 5 min) acidification (chemical, using GDL) and characterized in terms of denaturation degree of whey protein, particle size, textural properties, rheology and microstructure.

The model systems with NWP exhibited significantly larger particle sizes after heating and provided acid gels with higher firmness and viscosity, faster gelation and lower syneresis and a denser microstructure (see Figure 1 and 2). In contrast, microparticulated whey protein (MWP) appeared to only weakly interact with other proteins present and resulted in a protein network with low connectivity in the resulting gels.

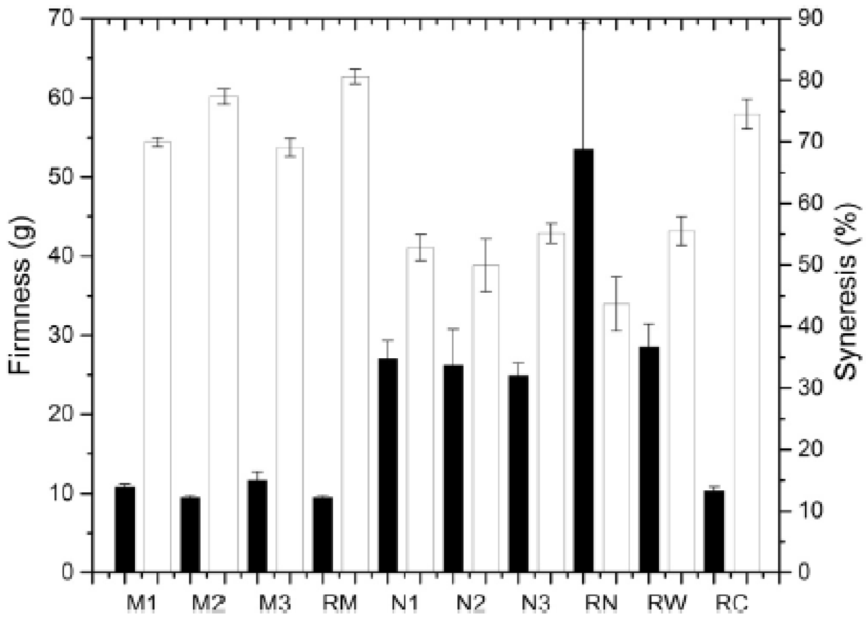

Figure 1: Firmness (black bars) and syneresis (white bars) of acidified milk model systems. Samples with **M** are made using microparticulated whey protein, whereas those marked with an **N** are made using nanoparticulated whey protein. From Liu et al., 2016, International Dairy Journal, 62, 43-52.

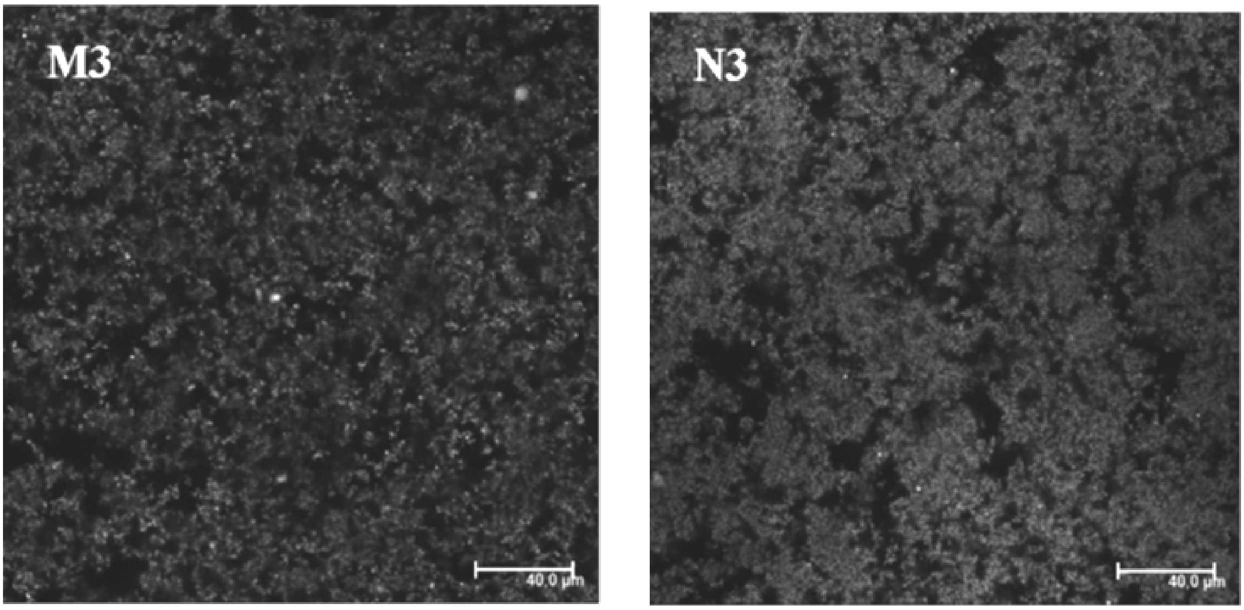

Figure 2: CLSM images of acidified milk model. Samples **M3** is made using microparticulated whey protein, whereas those marked with **N3** are made using nanoparticulated whey protein. From Liu et al., 2016, International Dairy Journal, 62, 43-52.

Part 2:

Following this, MWP and NWP were again investigated in an experiment specifically aimed at resolving the effect of the formation of disulphide bond during processing and how this affected the quality of fermented milk. Experiments were designed at laboratory scale and with use of a thiol-blocking agent (hence avoiding formation of disulphide bonds. Systems were characterized in terms of thiol groups, gel electrophoresis, particle size, and rheology during processing (homogenization, heat treatment and acidification). Results showed that formation of disulphide-linked structures in milk model systems was closely related to the increased particle size and rheological behavior of the gels. MWP enriched systems produced, upon acidification, weak protein networks and required the presence of whey protein (from isolate) to increase gel strength. Compared with the thiol-blocked systems, the non-blocked systems with NWP exhibited obvious increase in particle size and higher firmness (Figure 3), showing that NWP plays an important role in structure formation of acid milk gels through efficient thiol/disulphide interactions but also hydrophobic interactions with the other proteins present in milk are important for structure formation.

Part 3:

A number of experiments on the pure ingredients as well as on model milk systems were then made in order to more precisely clarify what interactions are dominant and how the added ingredients react with the rest of the milk matrix. This study was focused on systems with added NWP. In terms of surface hydrophobicity (S_0), the pure NWP system showed the highest, while micellar casein isolate and whey protein isolate showed low S_0 (Figure 4). Surface hydrophobicity increased with the abundance of NWP in the systems.

Results from this study also indicate that NWP self-associates above pH 5.5 and then further interacts with casein or casein/whey protein complexes. Apart from having high surface hydrophobicity, NWP also had the most negative zeta potential (i.e. charge) of the investigated systems, and it would appear that not only decrease in electrostatic repulsion, but other interactions such as hydrophobic interactions, are important in contributing to the high gelation pH of NWP observed (i.e. > pH 5.5).

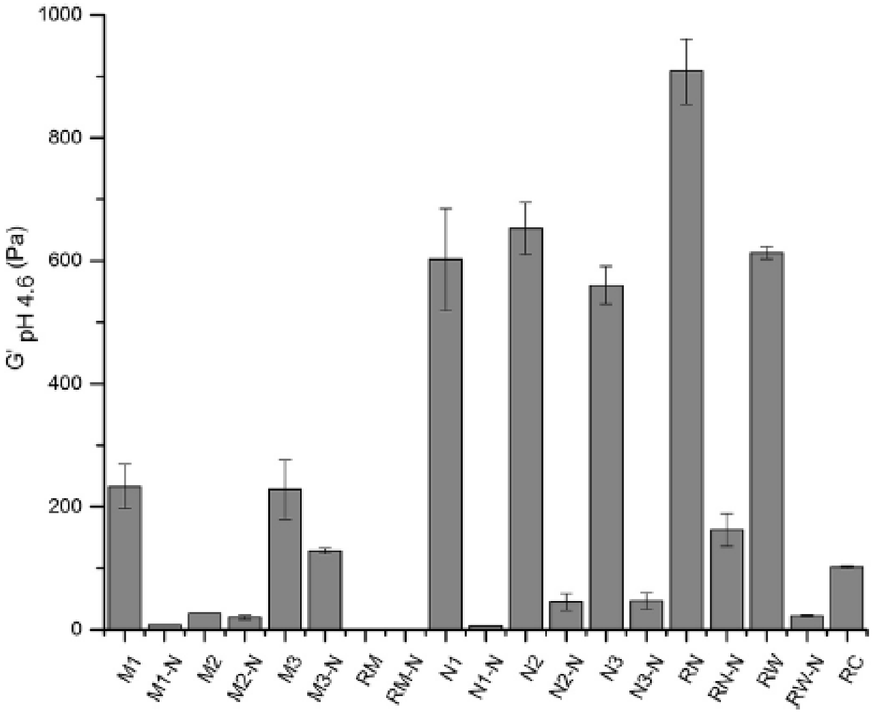

Figure 3: The gel strength (G') at pH 4.6 of acid gels made from heated milk model systems. Samples marked with an **M** are made using microparticulated whey protein, whereas those marked with **N** are made using nanoparticulated whey protein. The sample marked **RC** is made only using miceller casein isolate. From Liu et al., International Dairy Journal, 2016, 59, 1-9.

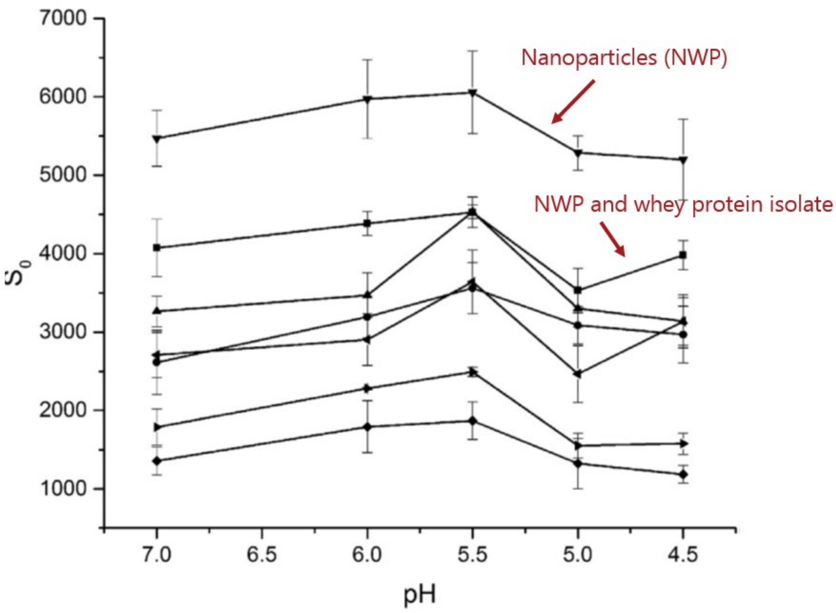

Figure 4: Surface hydrophobicity (S_0) of diluted milk model systems. Modified from Liu et al., International Dairy Journal, 74, 57-62.

A separate study was also set up to investigate the influence of ionic environment on the gelation behavior, i.e. comparing model systems made using addition of whey permeate and samples dialyzed against skim milk. It was found that the gelation behavior of samples in permeate was significantly different from that of the dialyzed samples even though the same conductivity was seen in all systems. MCI and MCI samples made with addition of MWP in permeate exhibited significantly longer gelation times and lower final gel strength (G' values) and also took longer to reach pH 4.6 than dialyzed samples. Rehydrated casein micelles could be influenced by the higher Ca^{2+} content in the permeate-added samples as a pH buffering effect will arise from the increased content of minerals in the permeate-added samples. These observations are of great importance when formulating milk model systems for future studies.

Part 4:

For the actual interaction studies, we set up a study in the final part of the project where Ca^{2+}-whey proteins interactions were thermodynamically characterized by titration of two different whey protein preparations (WPI or NWP) with calcium chloride using isothermal titration calorimetry (ITC). We were successful in establishing a method useful for characterizing calcium-binding in a system as complex and heterogeneous as NWP. Results showed that regardless of the whey protein product, the thermodynamic signal of Ca–whey protein interactions were endothermic in nature while the number of binding sites was found to be approximately 3 moles of added Ca^{2+} per protein for WPI and about 1.5 moles added Ca^{2+} per protein for NWP. WPI has a relative high amount of native whey proteins, while NWP contains mainly denatured whey proteins (as a consequence of processing) and this thus markedly affect its ability to bind calcium (as well as it physicochemical and functional properties).

The exploration of calcium-whey protein interaction in a whey protein powder product is highly relevant for producers of dairy ingredients as well as end-users of whey protein products. Whey protein products are used by the food industry to produce high-value nutritional products and knowing the thermodynamics of calcium binding to whey proteins can be relevant for fundamental understanding of the metal uptake, transport and delivery.

Conclusion:

The PROCOMP project has generated important novel knowledge for application of whey protein derived ingredients in fermented dairy products. It has been elucidated how characteristics of whey protein aggregates, especially the amount of thiol groups and the surface hydrophobicity affect interaction with other milk components. The project has also cast additional light on how these interactions determine performance in the final applications, with focus on fermented dairy products. The importance of hydrophobic and other non-covalent interactions for whey protein ingredient performance in dairy systems, previously somewhat neglected, has become evident. Thus, the obtained results provide new possibilities for design and development of whey protein aggregates as ingredients in dairy products.

In addition, the project has illustrated both advantages and limitations of using milk model systems and the obtained results will aid in design of future systems used in more fundamental interaction studies. PROCOMP has also shown the applicability of using Isothermal Titration Calorimetry for obtaining detailed information on the interaction of the various components with e.g. calcium in very complex and heterogeneous systems such as commercial whey protein ingredients. This shows promise for future work and could possibly be extended to include protein-protein interactions as was the original intention.

11. Deviations

11.1 Scientific

During the course of the project, we decided not to proceed with some of the more advanced analytical methods for interactions (SPR, QMB), since they were not readily adaptable for the complex ('dirty') systems we were looking at. Even our simplified milk models can cause issues in terms of results that are impossible to interpret. Hence, we focused late in the project on exploring the binding of calcium to the added whey protein ingredients (nano particulated whey protein, NWP) and beta-lactoglobulin, using ITC. We have thus not performed any experiments on protein-protein interactions with the advanced methods during the course of the project.

11.2 Financial

The budget was refitted to take the extension into account (see below) and we employed a dedicated post doc (1 year) to do the interaction studies. An additional post doc (Johnny Birch) has been employed for 2½ months from January 2017 to assist with the interaction studies.

11.3 Timetable

We were granted an initial extension until 31/8-2017 of the project in order to have a dedicated postdoc employed to do interaction studies based on the project results obtained so far. The post doc, Glykeria Koutina, was on maternity leave from 20th March 2017 and finalized the work when she returned (3rd April 2018). The project end-date was thus ultimo July 2018.

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

The PROCOMP project has generated important novel knowledge within application of whey protein derived ingredients in fermented dairy products. Based on these results is has become easier for producers of dairy based protein ingredients to tailor-make products and for producers for dairy products to apply the best ingredient.

The scientific relevance resides mainly in quantification of the importance of disulphide bonds and the hydrophobic interactions to final quality of fermented dairy products, but also the application of model systems is relevant for future projects. In addition, the ITC method for calcium binding will be used in future studies and the experience obtained also applied to protein-protein interaction studies. In fact, a newly approved DDRF project, Bespoke (Skræddersyede syrnede mejeriprodukter; nye strategier til forståelse af og kontrol med interaktioner mellem mælkeprotein-ingredienser og mælkens øvrige bestanddele under procesbehandling) directly builds on the experiences gained in PROCOMP and will integrate the experiences gained in the planning and execution of the experiments. Bespoke started 1st September 2018.

13. Communication and knowledge sharing about the project

Papers in international journals:

1. Guanchen Liu, Søren Bang Nielsen, Marianne Lund Lametsch, Jacob Holm Nielsen, Richard Ipsen 2016 Effects of disulfide bonds between added whey protein aggregates and other milk components on the rheological properties of acidified milk model systems, International Dairy Journal, 59, 1-9 http://dx.doi.org/10.1016/j.idairyj.2016.03.002
2. Guanchen Liu, Søren B. Nielsen, Patrizia Buldo, Richard Ipsen 2016 Effects of whey protein aggregates on textural and microstructural characteristics of acidified milk model systems, International Dairy Journal, 62, 43-52 http://dx.doi.org/10.1016/j.idairyj.2016.08.009
3. Guanchen Liu, Tanja C. Jæger, Søren B. Nielsen, Colin A. Ray, Richard Ipsen 2017 Interactions in heated milk model systems with different ratios of nanoparticulated whey protein at varying pH, International Dairy Journal, 74, 57-62 http://dx.doi.org/10.1016/j.idairyj.2016.12.010
4. Guanchen Liu, Tanja C. Jæger, Søren B. Nielsen, Colin A. Ray, Richard Ipsen 2018 Physicochemical properties of milk protein ingredients and their acid gelation behavior in different ionic environments, International Dairy Journal, 85, 16-20 https://doi.org/10.1016/j.idairyj.2018.04.012
5. Richard Ipsen 2017 Microparticulated whey proteins for improving dairy product texture, International Dairy Journal, 67, 73-79 http://dx.doi.org/10.1016/j.idairyj.2016.08.009
6. Johnny Birch, Glykeria Koutina, Emil Stender, Tanja C. Jæger, Søren B. Nielsen, Jacob H. Nielsen, Birte Svensson, Richard Ipsen, Interaction of calcium with whey protein isolate and nanoparticulated whey protein investigated by isothermal titration calorimetry, *To be submitted.*

Easily read papers:

1. Richard Ipsen, Guanchen Liu, Marie Tholstrup Greve, Jacob Holm Nielsen 2015 Valleprotein som ingrediens [Whey protein as ingredient], Mælkeritidende 128 (1), 6-7.

Student theses:

1. Guanchen Liu 2017, Interactions between milk protein ingredients and other milk components during processing, PhD thesis, University of Copenhagen.
2. Josefin Lundin 2015, Investigation of how changes in milk protein ingredient composition influence the properties of stirred type acidified milk gels, MSc Thesis, University of Copenhagen.

Oral presentations at scientific conferences, symposiums etc.:

1. Guanchen Liu: Effects of disulphide bonding between added whey protein aggregates and other milk components on the rheological properties of acidified milk model systems, 16th Food Colloids Conference (2016), Wageningen, NL
2. Guanchen Liu: Acid milk model systems with added nanoparticulated whey protein: interactions, structure and rheology', NIZO Dairy Conference - Asia Pacific 2016: Milk Protein Ingredients, Singapore
3. Richard Ipsen: "Functionality of milk protein ingredients in fermented dairy products as affected by processing", Invited talk at IDF World Dairy Summit, Belfast, 29/10-3/11 2017

Other:

1. Guanchen Liu, Richard Ipsen, Marie Greve 2015 Effects of whey protein particles on rheological and microstructural characteristics of an acidified milk model system Book of Abstracts ISFRS 2015: International symposium on food rheology and structure, Jun. 07 2015 - Jun. 11 2015, Zürich, Switzerland, Poster 7, p 138

14. Contribution to master and PhD education

As part of the project, one PhD has been educated and one M Sc student (Josefin Lundin) has done her thesis work directly in collaboration with the project. In addition, results from the project has been integrated in the teaching on the Dairy Specialization.

15. New contacts/projects

The project has provided the basis for a new project, Bespoke, which was granted funding by DDRF in 2016 and has now received co-founding (a PhD from Chinese Scholarship Council) so it was initiated in September 2018. The experience with model systems and analytical methods gained in PROCOMP has provided very valuable input to this project, which will further explore the interactions between added milk protein ingredients and the milk matrix.

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: _____________Signature, Project manager: ___________________________

Date: 17 October 2018 Signature, DDRF-representative: