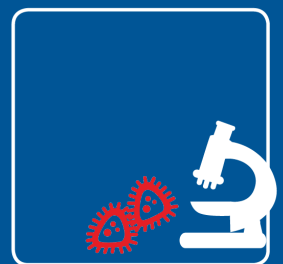


Milena Corredig:
FILTRATE – Forståelsen af centrale
processering parametre, for at designe nye,
forbedrede funktionelle mælkeprotein
koncentrater

FILTRATE – Understanding critical processing
parameters to design novel improved Functional
mILk protein concenTRATEs



Final report

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

1. Title of the project

Danish: Forståelsen af centrale processering parametre, for at designe nye, forbedrede funktionelle mælkeprotein koncentrat - FILTRATE

English: Understanding critical processing parameters to design novel improved functional milk protein concentrates

2. Project manager

Milena Corredig, Aarhus University, Agro Food Park 48, DK-8200 Aarhus N, mc@food.au.dk , +4522719132

3. Other project staff

Ozgenur Coskun, PhD student. Current position: AFI, ozcos@arlafoods.com

Norbert Raak, Post Doctoral Fellow, Current position, Assistant Professor, KU, nr@food.ku.dk

4. Sources of funding

Milk Levy Fund, Arla Foods Amba.

5. Project period

Project period with DDRF funding:

[January/2020, End, December/2022]

6. Project summary

Danish: Membranfiltrering har øget innovationen i mejerisektoren og åbnet nye muligheder for ingredienser med modificeret sammensætning og funktionalitet. Målet med dette projekt var at skabe en bedre forståelse på det supramolekylære niveau af ændringerne i mælkeproteiner og mere specifikt kasein, under opkoncentrering kombineret med diafiltration. Den nye viden, der er opnået i projekt, kan bruges til at sikre bedre definition af kvalitetsparametre og fastlæggelse af, hvilke procesbetingelser der er kritiske for ændringer i funktionaliteten.

Igennem projektet er en række studier udført ved parallelt at koncentrere ved både anvendelse af mikrofiltrering (MF) og ultrafiltrering (UF) og med forskellige grad af diafiltration (DF) for herved at forstå den rolle som tilstedeværelsen af valleproteiner spiller for stabilitet og gele-egenskaber af koncentratene. En gennemgående evaluering af sammensætningen af både opløselige og kolloidale komponenter ved avancerede analytiske teknikker, blev kombineret med en dybtgående undersøgelse af kaseinets dissociation under koncentration. Herved er der opnået en mere klar identifikation af den rolle, som opløseligt materiale spiller i disse koncentrat. Ved systematisk at udfører UF og MF under forskellige DF-betingelser er det for første gang vist at graden af valleprotein permeation påvirker interpartiku-

lære interaktioner mellem kaseinmiceller og at det har konsekvenser for deres gelering og varmemestabilitet. Ved udvaskning af valleproteiner var kaseinmicellerne i koncentratene mere intakte og havde et højere indhold af kolloidalt calcium pr. kasein sammenlignet med koncentrat med valleproteiner.

Derved er konklusionen fra dette projekt at den overordnede sammensætning ikke er tilstrækkelig til at forudsige den teknologiske kvalitet af mælkeproteinkoncentrater. Det er vist at en detaljeret forståelse af den kolloidale struktur af kaseinmiceller, baseret på serumsammensætning, er afgørende for at forstå deres funktionelle egenskaber. Igennem dette projekt er der også etablerede en nye værktøjskasse til at evaluere ændringer i den interne struktur af kaseinmiceller, ved brug af X-ray scattering. Det åbner helt nye muligheder for i fremtiden bedre at forstå strukturelle ændringer selv under milde procesforhold.

English: Membrane filtration in dairy technology has boosted innovation, opening new opportunities for the creation of dairy ingredients with modified composition and functionality. This project aimed at a better understanding at the supramolecular level of the changes occurring to caseins during concentration, in combination with diafiltration. The new knowledge derived from such studies should lead to better defining quality parameters and determining which processing conditions may be critical to modifications in technological functionality. Studies were conducted in parallel using microfiltration and ultrafiltration, with different extents of diafiltration, to evaluate the role played by the presence of whey proteins in imparting stability and gelling properties to the concentrates. Advanced analytical techniques evaluated the composition of the soluble and colloidal phase, and an in-depth study of casein dissociation during concentration led to clearly identifying the role played by soluble material in these concentrates. For the first time, in this study, we systematically compared UF and MF under different DF conditions and concluded that the permeation of whey proteins affected the inter-particle interactions between the casein micelles, with consequences on their gelation and heat stability. The depletion of whey proteins affected the casein micelle dissociation and colloidal calcium phosphate solubilization. Whey protein depleted micelles were more intact, contained a higher amount of colloidal calcium per casein, and showed less casein micelle dissociation compared to the milk concentrates still containing whey proteins, which were processed with UF membranes. In conclusion, this study demonstrated that only the total composition was not sufficient to predict the technological quality of milk protein concentrates and that a detailed understanding of the colloidal structure of casein micelles based on their serum composition is critical to their functional properties. This project established a new toolbox to evaluate changes in the internal structure of the casein micelles, using small angle X-ray scattering, enabling both academics and industrial R&D to determine structural changes in these protein particles even in mild processing conditions.

7. Project aim

Danish:

Målet med dette forskningsprojekt var at bestemme hvilke behandlingsparametre, der er kritiske for ændringer på et supramolekylært niveau i kaseinmicellernes struktur, og at beskrive kvalitetsparametre, der kan bruges til at forudsige ændringer i kaseinmicellernes funktionalitet. Baseret herpå var der i projektet følgende specifikke mål:

- 1) At opnå en bedre forståelse for den opløselige proteinfraktion i koncentratene.
- 2) Skabe en dybdegående forståelse af varmemestabiliteten og chymosin inducerede gelerings-egenskaber af koncentratene.
- 3) Vurder potentiel udnyttelse af koncentrat af interesse.

English:

The research aimed to determine which processing parameters are critical to cause changes at a *supramolecular level* to the structure of the casein micelles, and to describe quality parameters able to predict changes in casein micelles functionality. With this aim, the research had the following specific objectives:

- 1) To understand the state of the soluble protein fraction in the concentrates.

- 2) Develop a comprehensive understanding of the heat stability and the ability of the concentrates to gel with chymosin.
- 3) Evaluate potential utilization of concentrates of interest.

8. Background for the project

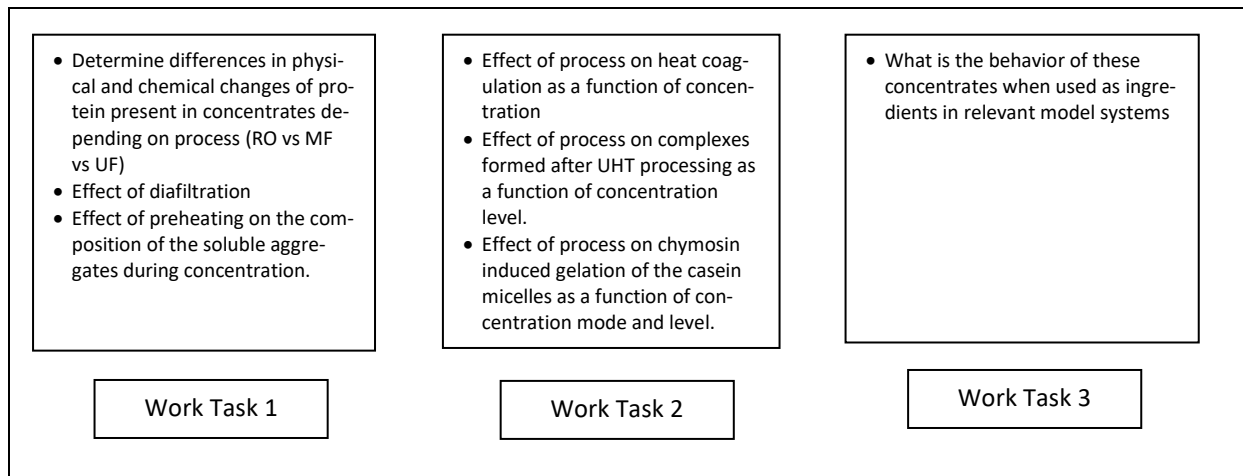
Casein micelles are often described as sponges, with a behavior during concentration that can be predicted by thinking of them as hard spheres being compressed in space, without consequences to their structure and function. With this thinking, concentrates are prepared and then utilized as ingredients in dairy and food applications, with the assumption that the casein micelles will show the same properties observed for skim milk. It is well known that the micelles are an assembly of various caseins, with a high ratio of water to protein (about 3.5 mL/g) and with areas more or less dense with protein, allowing for water channels to exist within the inner structure. However, their structural integrity remains in question. Caseins are indeed rheomorphic proteins, which means that they quickly adapt their structure to the environmental conditions.

Membrane filtration is a widespread practice in dairy technology, as it is a gentle process to selectively concentrate milk components. During concentration, the milk is separated into a retentate phase (concentrate) and a permeate phase which is transmitted through the membrane. Depending on the pore size and chemistry of the membrane, it is possible to selectively concentrate proteins in the retentates, and with that, change the composition of the soluble fraction. Although it is generally recognized that the main proteins in milk fully maintain their structure and functionality during this process, this is not the case for their supramolecular structures, which may be impacted at critical concentrations, or when water is added. This project focused on the critical processing steps that affect the protein organization, and the formation of new protein aggregate structures which could modify the functional properties of the retentates. The knowledge created will be used to control quality and create new functional ingredients from milk.

9. Sub-activities in the entire project period

The project underpinning hypothesis was that any changes in the environmental conditions during membrane concentration, combined with changes of their interparticle distance, will result in a rearrangement of the casein micelles supramolecular organization. Although investigating structural changes may be a challenge, observing differences in the colloidal behavior may be easier, as their reactivity also changes. Any modifications in the concentrations and ratios of soluble ions (and especially the equilibrium between soluble and colloidal ions) during the process of concentration will not only affect the internal rearrangements of the caseins, but also the state of the proteins in solution and their aggregation. Serum proteins are prone to interact with themselves as well as with colloidal and soluble caseins when subjected to heating treatment, and the complexes form depend not only on time and temperature but also on the solute concentration and pH of the soluble phase. There is a lack of knowledge on the presence and composition of these whey protein aggregates in milk protein concentrates, especially as a function of processing parameters (specifically, membrane selectivity, composition changes, temperature, diafiltration, pre-heating).

Project content



Time plan for the project period January 2020 to December 2022, including milestones and responsibilities

	2020	2021	2022
WT1. M1: a comprehensive description of molecular details of the soluble complexes formed depending on pre-processing treatments during concentration using RO, UF, MF and extent of diafiltration. PhD student, Post Doctoral Fellow, MC, LW, LBL, Arla			
WT2. M2: a comprehensive description of molecular details of changes occurring to concentrates after heating. Post Doctoral Fellow, PhD MC, LBL, LW, Arla.			
WT2. M3: compare heating complexes generated in concentrates compared to those concentrated from heated milk. PhD, MC, Arla			
WT2. M4: a mapping of stability of UHT concentrates depending on processing history parameters (preheat temperature, concentration type). PhD, MC, LW, Arla			
WT2. M5: testing chymosin induced gelation of various milk concentrates obtained by RO, UF, MF, as a function of extent of diafiltration and composition of the soluble fraction. Post Doc, MC, LW			
WT3. M6: testing of relevant model systems using UHT, shelf-life PhD, MC, Arla.			
WT3. M7: gelation of concentrated milk added to skim milk PhD, MC, LW			

10. Deviations

No deviations are reported, however testing of relevant model systems was carried out in the pilot plant in AU Food as well as Kold college and not at ARLA Amba.

11. Project results

Outcome of this research is a detailed understanding of the processing parameters affecting the functional behavior of milk protein in concentrates and determining principles to control the quality or develop new ingredients using processing conditions targeted to functionality.

1. Understand the state of the soluble protein fraction in the concentrates

- Soluble fractions of the concentrates were characterized extensively using several techniques such as SEC, RP-HPLC, SAXS, ICP-MS at 2x and 4x concentration factors and diafiltered with UF permeate and water. In addition, ultrafiltered and microfiltered concentrates were compared in order to understand the effect of whey protein concentration on the formation of soluble aggregates.
- Whey protein depleted concentrates had lower soluble aggregates compared to ones that contains whey proteins
- At 4x concentration factor, concentrates had higher soluble aggregates compared to 2x concentrated ones in both ultrafiltered and microfiltered samples.
- Diafiltration with water resulted in more dissociation of casein micelles, which led to higher soluble proteins.
- Colloidal calcium phosphate solubilization reaches a plateau at 2x concentration.
- Soluble caseins did not differ between low and high diafiltered ultrafiltered concentrates.
- By a more detailed understanding of the role played by the soluble aggregates, it was possible to create a model on absolute scale of the structure of casein micelles to predict small angle X-ray scattering data, creating a powerful toolbox for probing, structural changes during processing.
- Changing the diafiltration milieu from permeate to water, showed no changes in the transmission of whey proteins during microfiltration.
- Diafiltration with water decreased the level of permeable calcium, but not the soluble calcium, pointing to the association of calcium with non-sedimentable proteins.
- Small-angle scattering patterns of the casein micelles revealed changes in their internal structure by DF with water, in contrast, the structure of the casein micelles was much less affected upon DF with UF permeate.
- Concentration of casein micelles using water as the DF medium to reach high concentrations affects the structure and integrity of casein micelles, possibly with consequences on the techno-functional properties of the casein ingredients.

2. Develop a comprehensive understanding of the heat stability and the ability of the concentrates to gel with chymosin and 3. Evaluate potential applications for these modified concentrates

- Rennet- and acid- induced gelation as well as heat stability of the concentrates were investigated with rheological techniques to understand the colloidal state of casein micelles.
- Enzymatic stage of rennet-induced gelation did not differ between the ultrafiltered and microfiltered samples that are produced by low and high diafiltration.
- Control samples were compared with skim milk and non diafiltered concentrates and there was no difference in the enzymatic stage of rennet-induced gelation which indicates that hydrophilic portion of the casein micelle is still on the outside of the micelle

- However, rheological properties of the concentrates were different. 4x concentrated, non diafiltered concentrates gelled at the same time compared to skim milk, but they formed stronger gel, probably because of the increased protein-protein interactions.
- Low diafiltered concentrates, again gelled at the same time compared to skim milk but they had significantly lower storage modulus compared to skim milk, which attributed to the lower soluble calcium level.
- Interestingly, high diafiltered concentrates had a higher storage modulus compared to skim milk. In addition, whey protein depleted concentrates (microfiltered) had even higher storage modulus compared to ultrafiltered concentrates, which indicates that the whey protein and at some extent β -casein depletion affected the colloidal properties of the casein micelles, which then led to a different type of rennet-gels.
- Acid gelation pH increased with diafiltration extent and microfiltered concentrates, had a higher gelation pH compared to ultrafiltered concentrates, which again shows the effect of whey protein and β -casein depletion affected on the colloidal properties of casein micelles
- Concentrates were heated using a starch pasting rheometer cell at 85 °C for 1 min to simulate High temperature short time (HTST) pasteurization process. Microfiltered concentrates showed a lower viscosity compared to ultrafiltered concentrates which shows the effect of whey protein depletion as both of the concentrates had comparable colloidal caseins, calcium and phosphate. Hydrodynamic size reduced with heating, however, this reduction was more pronounced in microfiltered samples compared to ultrafiltered concentrates.
- Pilot-scale microfiltered concentrates were then produced in Kold College at different trans membrane pressure (TMP) and diafiltration extents. These concentrates were then heated at pilot scale UHT system (OMVE, Netherlands).
- The viscosity reduced in low diafiltered microfiltered concentrates, however, as the extent of whey protein depletion increased, viscosity did not change with heating, indicating that whey protein depletion resulted in more stable casein micelles after heating.

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

Milk protein concentrates obtained using membrane filtration are employed as ingredients in increasingly sophisticated forms. In the market space, while in the past most concentrates were classified by protein content (i.e. ratio of protein to solids), more recently, there has been an increased effort to market these ingredients targeting functional properties, which are the result of careful modification in composition or processing history.

Outcome of this research is a detailed understanding of the processing parameters affecting the functional behavior of milk protein in concentrates, and determine principles to control the quality or develop new ingredients using processing conditions targeted to functionality.

We now have established means to create a comprehensive mapping of the colloidal behavior of the casein micelles in concentrated systems as affected by post processing.

Amongst the measurable outcomes of the project are referred publications and presentations at international conferences

13. Communication and knowledge sharing about the project

Papers in international journals:

1. Coşkun, O., Raak, N., Corredig, M. 2023. *Heat induced interactions in whey protein depleted milk concentrates: comparison of ultrafiltration and microfiltration.* Food Hydrocolloids, 137, 108354.
2. Coşkun, O., Corredig, M. 2023 *Cold ultrafiltered and microfiltered milk retentates. A systematic comparison of the effects of compositional differences on their gelation functionality.* Journal of Dairy Science, 106,3123-3136.

3. Raak, N, Pedersen, JS, Corredig, M. 2023 *Structural changes of colloidal casein micelles and their dependence on diafiltration conditions*. Colloids and Surfaces A: Physicochemical and Engineering Aspects. doi:<https://doi.org/10.1016/j.colsurfa.2023.131580>.
4. Raak, N., Coskun, O., Corredig, M. 2023 *Transmembrane pressure during micro- and diafiltration of milk affects the release of non-sedimentable caseins*. Foods, 12, 2234. <https://doi.org/10.3390/foods12112234>.
5. Coşkun, Ö., Wiking, L., Rahimi Yazdi, S., Corredig, M. 2022. *Molecular details of the formation of soluble aggregates during ultrafiltration or microfiltration combined with diafiltration of skim milk*, Food Hydrocolloids, Volume 124, 2022, 107244.
6. Pedersen JS, Møller TL, Raak N, Corredig M 2022 *A model on absolute scale for the small-angle x-ray scattering from bovine casein micelles*. Soft Matter, 18, 8613–8625.
7. Raak N, Corredig M 2022 *Kinetic aspects of casein micelle cross-linking by transglutaminase at different volume fractions*. Food Hydrocolloids, 128, 107603.

Easily read papers:

8. How much can I squeeze milk proteins? – Mælkeritidende. 2021, nr 5.

Student theses:

1. PhD Thesis: Coskun, O. (2022). A colloidal perspective of the changes occurring to milk protein concentrates during membrane filtration combined with diafiltration.

Oral presentations at scientific conferences, symposiums etc.:

1. Corredig, M. 2024. Casein micelles structure and processing functionality. American Dairy Science Association Symposium Presentation, West Palm Beach, Florida.
2. Pedersen JS, Møller TL, Raak N, Corredig M 2022 A more complete model on absolute scale for the scattering from bovine casein micelles. 18th Nordic Workshop on Scattering from Soft Matter, online.
3. Corredig M., Raak N., Mardal F., Coşkun Ö., 2022 “Tailoring functional properties of milk protein concentrates using membrane filtration” 5th Meeting Forum for Membrane Filtration Technology in Food Processing, Aalborg, Denmark, (Oral Presentation).
4. Raak N, Pedersen JS, Corredig M (2022) Understanding the impact of diafiltration on the internal structure of casein micelles using scattering techniques. 18th Food Colloids Conference, online.
5. Coskun, O., Wiking, L., Yazdi, S.R, Corredig, M. 2021. Understanding the dynamics of molecular dissociation during membrane filtration of milk concentrates. American Dairy Science Association Annual Meeting. (Finalist at the graduate oral presentation competition).
6. Raak N, Rahimi Yazdi S, Pedersen JS, Corredig M. 2021 Structural details of casein micelle suspensions obtained by microfiltration combined with different diafiltration conditions. 12th NIZO Dairy Conference, online.
7. Coşkun Ö., Wiking L., Rahimi-Yazdi S., Corredig M., 2021 Soluble complexes formation during the microfiltration combined with diafiltration of skim milk. Dairy Science and Technology Symposium, Virtual Meeting, 2021 (Oral Presentation).

Other:

1. Coşkun Ö., Wiking L., Valentin R., Corredig M., “Gelation behavior of milk protein isolate and micellar casein concentrate produced by micro- or ultrafiltration in combination with various extent of diafiltration” 5th Food Structure and Functionality Symposium, Cork, Ireland, 2022 (Poster Presentation).

Various presentations at the DDRF meetings, and internally at ARLA food Amba.

14. Contribution to master and PhD education

One PhD student educated within the project.

15. New contacts/projects

The activities of this project have established research leadership in the field. Other projects spearheaded by Filtrate are "SURFMILK" also funded by DDRF and Milk foundation, and more recently, "QforCAS".