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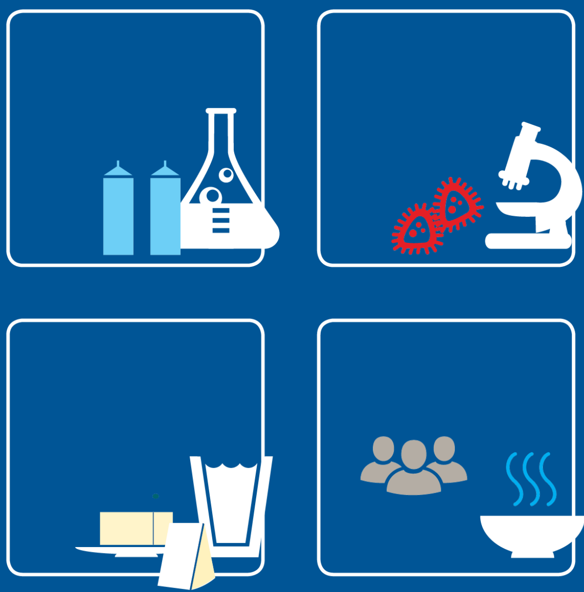
Milena Corredig:

PROFLEX – Strukturel design af fødevaremodeller med mælkeproteiner for en flexitarkost

PROFLEX – Mastering structure design in model foods containing dairy proteins for flexitarian diets



Mejeribrugets
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Final report

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1. Title of the project

Danish: PROFLEX: Strukturel design af fødevaremodeller med mælkeproteiner for en flexitarkost.

English: PROFLEX: Mastering structure design in model foods containing dairy proteins for flexitarian diets.

2. Project manager

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4. Sources of funding

Milk Levy Fund, Arla Food ingredients.

5. Project period

Project period with DDRF funding: January/2021, December/2023

6. Project summary

Danish:

Målet med dette projekt var at forstå, hvordan vi kan kontrollere egenskaberne af emulsionsbaserede fødevarer indeholdende blandinger af plante og mælkeproteiner. Projektet dækker både mekanistiske studier af grænseflader, model systemer og test i applikationer. Resultaterne illustrerer, at en sådan holistisk tilgang er nødvendig for at forstå disse komplekse systemer. Både for ærte-valle og lupin-valle protein stabiliserede emulsioner er det påvist at hvornår og i hvilken rækkefælge disse tilsættes har betydning for sammensætningen af grænsefladen og at dette igen kan påvirke egenskaber af emulsionen.

Det er også påvist at graden af aggregering i de planteprotein blandinger der anvendes, har stor betydning for deres stabilisering af emulsioner. Dette er også gældende for blandede ærte-valle- og lupin-valle-grænseflader. Dette viser

at det er nødvendigt at forstå udgangsmaterialet for at kunne forudsige stabiliseringen af emulsioner i og med at både sammensætningen og aggregering af planteproteiningredienser fra bælgfrugter kan variere væsentligt.

I projektet er det også vist at resultaterne fra simple emulsioner også korrelerer med opførsels i mere komplekse gelerede emulsionsstrukturer, et mere realistisk modulsystem for mejeriprodukter. Det var muligt at manipulere strukturen af disse systemer ved at modulere interaktionerne i plante-valleprotein blandinger, både ved grænsefladen og de overordnede egenskaber. De grundlæggende mekanismer, der blev observeret, gjaldt også, i studier hvor vi har anvendt procesbetingelser af industriel relevans. Som forventet skyldtes forskellene i de endelige gelstrukturer den dynamiske opvarmning anvendt under industrielle forhold, hvilket understreger vigtigheden af at bruge relevante eksperimentelle betingelser under studier i laboratorie skala.

Overordnet viser resultaterne af dette projekt at planteproteiner ikke kun kan inkluderes i mejeribaserede fødevarer for at øge produktets overordnede bæredygtighed, men faktisk giver en unik mulighed for at kontrollere de strukturelle egenskaber og teksturen, hvilket fører til nye anvendelser og produkter. Plante-valleproteinblandinger i fødevarer kan derfor ses som en innovativ, bæredygtig og tilpasningsdygtig løsning til udvikling af fremtidige fødevarer.

English:

The following summary is slightly modified from the conclusion of the PhD thesis output of this project (Grasberger, 2023).

The aim of this project was to learn how to employ mixed plant-dairy protein isolates in emulsion-based foods to best utilize potential synergies between them. The research included fundamental studies of protein interactions at the interface and extending our knowledge to real-world applications, with results illustrating that such a holistic approach is needed to understand these complex systems. Results demonstrated how the point and order of protein addition can be used to achieve various emulsion properties in mixed pea-whey and lupin-whey protein stabilized emulsions. The bulk emulsion properties could be linked to the interfacial behavior of mixed plant-whey model interfaces and revealed differences in the interfacial structures depending on the order of protein addition. This has important consequences for food product development.

The colloidal state of the proteins affected the emulsifying properties. The aggregation state of the plant proteins not only impacted the behavior of pea protein interfaces but also that of mixed pea-whey and lupin-whey interfaces. We demonstrated that the ability to predict interfacial properties requires an understanding not only of the composition of the various protein fractions but also of the various protein assemblies and aggregation states.

The broader impact of this work is shown by its relevance in both bulk fluid emulsions and gelled emulsions. It was possible to manipulate the structure of these systems through modulating the interactions in plant-whey protein mixtures, both in the bulk and at the interface. The fundamental mechanisms observed also applied when we expanded the study of these systems using processing conditions of industrial relevance.

In conclusion, this work demonstrates that plant proteins can not only be used in dairy-based foods to increase the overall sustainability of the product, but also as an opportunity to tailor the final textural and structural attributes, leading to novel applications —presenting the use of plant-whey protein mixtures in food as an innovative, sustainable, and adaptable solution for the development of future foods.

7. Project aim

Danish: I PROFLEX anvendes en 'soft matter' dvs. 'blød struktur' tilgang til at koble en grundlæggende viden om protein-protein interaktioner, olie-i-vand emulsioner og effekten af processering til at studere nye fødevareapplikationer, der indeholder proteiner fra hhv. mælk og plantekilder. Formålet med PROFLEX er at opnå forståelse af, på forskellige størrelsesskalaer, hvordan strukturdannelsen sker i multifase fødevarematricer, som indeholder både mælkeproteiner og planteproteiner under realistiske forhold in situ. Resultaterne vil bibringe opdagelse af nye hydrokolloidlignende funktionaliteter af disse proteinblandinger og heraf vil nye principper kunne udledes for, hvordan man kan designe geler og emulsioner som indeholder proteiner fra blandede kilder.

English: We are proposing a soft matter approach to link the fundamental knowledge of protein-protein interactions, oil in water emulsion systems and processing effects to study novel food applications containing proteins from dairy and plant sources. Objective of this work is to understand, at various length scales, the structure formation of multi-phase food matrices containing both dairy proteins and plant proteins, under realistic conditions, in situ. Results will bring to the discovery of new hydrocolloids-like functionalities of protein blends and will derive new principles on how to design emulsions and gels containing proteins of mixed sources.

8. Background for the project

The following background is slightly modified from the PhD thesis output of this project (Grasberger, 2023).

Although plant-based food consumption has experienced a noteworthy surge in recent years, driven by heightened consumer awareness of their benefits and the growing number of flexitarian consumers (Cliceri *et al.*, 2018; Taufik *et al.*, 2019; Aschemann-Witzel *et al.*, 2021), it is not enough to rely on changing consumer behavior, as this is a slow cultural process (Henchion *et al.*, 2017). It is urgent to find a viable sustainable solution already now (United Nations General Assembly, 2015). To achieve a more sustainable food system, recent models suggest a more holistic approach, where the most sustainable diets include a small percentage of animal protein for optimal land use and high levels of biodiversity (Van Kernebeek *et al.*, 2016; Van Zanten *et al.*, 2018). Additionally, encouraging a sustainable diet does not only mean to address climate change concerns, but as defined by the FAO (FAO, 2010),

"Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy; while optimizing natural and human resources."

Therefore, one possible solution, and the aim of this project, is to combine plant and dairy proteins to make novel food products. Partially replacing dairy proteins with plant proteins would allow for more sustainable dairy alternatives with similar textures and taste to current dairy products while still maintaining a high protein quality.

The combination of plant and milk proteins has been researched since the 1970s as a way to fortify plant-based products by increasing their nutritional value or to reduce costs of premium products (Thompson, 1977; Craig, 1979; Morr, 1979; El-Neshawy, Farahat and Wahbah, 1988). However, in depth research in this area has been so far missing (Alves and Tavares, 2019), and there are still several challenges to promote the transition to using plant proteins in dairy formulations.

The current processes and formulations for dairy-based products are well fit and optimized to the techno-functional properties of dairy ingredients. Simply adding plant-derived proteins to dairy processes does not allow for optimal plant protein functionality, and this can negatively affect the final product properties. Reinventing the current processes is expensive and difficult; hence, the focus of the project is to understand how plant proteins can be included in the current systems and ways to modify the processes to achieve novel plant-dairy protein foods of high quality. While the work in this project provides relevant knowledge to industry on how the process can be modified to take advantage of the properties of both plant and dairy ingredients, the adoption of plant-dairy hybrid foods by consumers also requires clear communication between industry and consumers underlining the benefits of hybrid products.

9. Sub-activities in the entire project period

Overview of tasks in the project period. Task 4 was planned but not conducted as marked by strikethrough text.

Tasks	2021				2022				2023			
Task 1: M1.1 finish screening; M1.2 characterization of aggregates; M1.3 selection of 3 best potential blend		1		3						2		
Task 2: M2.1 studies of the dynamics at the interface using drop tensiometry. M2.2 finished emulsion characterization with selected blends. M2.3 finished microstructure studies of the emulsions.					1		2, 3					
Task 3: M3.1 destabilization of emulsions and gelation studies, for two selected sytems. M3.2 Rheological and microstructural characterization of gelled emulsions formed on pilot scale									1		2	
~~Task 4:~~ ~~M4.1 *in vitro* digestion studies on two selected emulsions~~												
Report writing and PhD-thesis hand-in												

10. Deviations

The last part of the project related to preliminary studies of de-structuring during gastrointestinal transit under task 4 were not carried out so far. Due to the very interesting results on the mixes and their interactions, we decided to dedicate more time to pilot plant model systems in collaboration with the industry partner, AFI. This brought some very nice results using a model system, that albeit pre-competitive in nature, will show how it is possible to create a sustainable matrix based on dairy proteins but also containing plant proteins, and with appealing taste and texture. Digestion studies on such systems may be carried out in future projects, once potential industrial interest for dairy blends becomes more clear, as the results would be very system specific.

11. Project results

The objective of this project was to explore the complex relationship at various length scales between the various components present in selected protein blends with milk proteins, their distributions in a multiphase system containing oil droplets, and their ability to form structures in emulsions and gels. The results of the work are summarized below under each of the aims listed in the project proposal.

<u>**Aim 1.**</u> **Screening of blends and characterization of the aggregates. M1.1** Various commercial pea and lupin proteins were screened for differences in their protein composition, solubility, particle size, microstructure, z-potential, emulsifying ability (creaming), gelling ability and interfacial tension. **M1.2** The ingredient properties were also screened following homogenization to determine the effect of a pre-homogenization step on the aggregates. **M1.3** Based on the results, two ingredients (one pea and one lupin ingredient) were selected. The selected samples were chosen because they were not fully denatured and had an average solubility compared to the other commercial ingredients and thus were representative of the current commercially available proteins on the market.

<u>**Aim 2.**</u> **Analysis of protein distribution in mixed interfaces, and adsorption dynamics. M2.1 and 2.2** Both combinations of pea and whey (Grasberger *et al.*, 2022) and lupin and whey (Grasberger, Hammershøj and Corredig, 2023) were studied at model interfaces using pendant drop tensiometry and in bulk emulsions. The competitive adsorption of the mixed protein systems was compared by adding the proteins either simultaneously or sequentially. Characterization of the interfacial composition of the bulk emulsions showed significant differences depending on the point

(simultaneous or sequential) and order of protein addition (M2.2). The same mixtures were studied at model interfaces using pendant drop tensiometry to determine the differences in the protein adsorption kinetics and displacement depending on the order of protein addition (M2.1). These results could be linked with the bulk emulsion results. The results showed a good emulsifying ability of both pea and lupin protein at the interface, which was comparable to that of whey protein. Furthermore, the order of addition played a significant role in the interfacial properties and thereby can be used to fine-tune emulsion properties. **M2.3** TEM imaging of the mixed lupin-whey emulsions showed that the flocculation of oil droplets is a general behavior for emulsions formed with legume proteins containing aggregates, such as in this case with the industrially processed lupin proteins (Grasberger, Hammershøj and Corredig, 2023).

AFM was also used to study the microstructure of pea proteins at the interface in an attempt to link the colloidal state of the pea proteins to their interfacial behavior and structure (K. F. Grasberger *et al.*, 2024). This was important to understand as it plays a role in how the pea proteins behave in combination with whey protein, particularly in understanding the role of the different aggregation states of plant proteins at the interface. This is important for our understanding of their properties in emulsions when industrially-processed/commercial proteins are used where the proteins exist both as monomers, colloidal aggregates, and insoluble particles.

<u>Aim 3.</u> Aggregation and gel formation of multiphase systems. M3.1 Building on the outcomes of aim 2, the results of aim 3 showed how mixed plant-whey interfaces could be used within a gelled whey protein matrix to form emulsion-filled gels on both lab and pilot scale. For this work, mixtures of lupin and whey protein were used. Differences in the gelation behavior and final gel structure were observed depending on the ratio of lupin to whey protein at the interface (Grasberger, Hammershøj and Corredig, 2024). By changing the interfacial composition, the extent and type of interactions between the interfacial proteins and the gelled whey protein matrix could be modified. When lupin was used to stabilize the emulsions, the whey protein matrix interacted with interfacial lupin proteins primarily via non-covalent interactions. Alternatively, when whey protein was present at the interface, the interfacial whey proteins and whey proteins present in the matrix interacted via disulfide bonding. This resulted in more interspersed droplets and allowed for increased reinforcement of the gel matrix and thereby stiffer gels. **M3.2** Due to the promising results of the gelled emulsions, the same mixtures were evaluated on pilot scale in collaboration with industry partners (Arla Foods Ingredients) using a plate heat exchanger to understand the role of shear during heating like that encountered in industrial processes (K. Grasberger *et al.*, 2024). These results showed that the dynamic conditions experienced on industrial scale have a significant impact on the final gel properties, as the gels formed at pilot scale were stiffer in comparison to those formed under static conditions in the rheometer. This shows the importance of studying such systems under relevant processing conditions. Still similar to the systems in Grasberger, Hammershøj, et al., (2024) softer gels with less uniform structures were formed oil droplets were stabilized with lupin proteins.

<u>Aim 4.</u> Preliminary studies of de-structuring during gastrointestinal transit. These systems were not studied as it was decided to prioritize the studies on the gelled emulsions to show the robustness of the novel approach used to structure emulsions gels with plant-whey mixtures for commercial applications. This allowed for a more in-depth characterization of the droplet interface and whey protein matrix interactions, which provides a solid foundation for future studies on the de-structuring of these matrices during gastrointestinal transit and how the breakdown during digestion is affected by these interactions.

In conclusion, this work highlights the potential of plant-whey protein mixtures in emulsion-based foods, facilitated by a thorough understanding of plant protein ingredients. The changes in emulsion structure resulting from different mixing methods of plant and whey proteins are supported by a fundamental understanding of interfacial properties and interactions at the interface. Overall, this work opens up an opportunity for industry to design novel high-protein foods using blends of plant and whey protein.

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

The project results are scientifically relevant as they advance the understanding of plant-whey protein interactions in emulsions and gels, offering new insights into protein adsorption dynamics, interfacial properties, and gel formation. Creating food products in the future will rely on the delicate balance between sustainability, nutritional properties, affordability and appeal. Dairy/plant-based protein blends are a possible solution for this dilemma. It was demonstrated how plant-dairy protein mixed systems can not only be used in dairy-based foods to increase the overall sustainability of the product, but also as an opportunity to tailor the final textural and structural attributes, leading to novel applications. Socially, the research aligns with the growing demand for sustainable, protein-dense foods, providing a balanced approach that caters to flexitarian consumers while supporting environmental goals. For the dairy industry, the findings present opportunities to innovate by developing high-protein, plant-dairy hybrid products that meet consumer trends and optimize industrial processing. Future research could explore digestion behavior, optimization of protein blends for other applications like beverages, incorporation of other plant protein sources, such as from oilseeds, consumer acceptance studies, and sustainability assessments to further enhance the impact of this work.

13. Communication and knowledge sharing about the project

Papers in international journals:

- Grasberger, K., Sunds, A. V., Sanggaard, K. W., Hammershøj, M., & Corredig, M. (2022). Behavior of mixed pea-whey protein at interfaces and in bulk oil-in-water emulsions. *Innovative Food Science & Emerging Technologies*, *81*(August), 103136. https://doi.org/10.1016/j.ifset.2022.103136
- Grasberger, K., Hammershøj, M., & Corredig, M. (2023). Stability and viscoelastic properties of mixed lupin-whey protein at oil-water interfaces depend on mixing sequence. *Food Hydrocolloids*, *138*(December 2022), 108485. https://doi.org/10.1016/j.foodhyd.2023.108485
- Grasberger, K. F., Lund, F. W., Simonsen, A. C., Hammershøj, M., Fischer, P., & Corredig, M. (2024). Role of the pea protein aggregation state on their interfacial properties. *Journal of Colloid and Interface Science*, *658*(September 2023), 156–166. https://doi.org/10.1016/j.jcis.2023.12.068
- Grasberger, K., Hammershøj, M., & Corredig, M. (2024). Lupin protein-stabilized oil droplets contribute to structuring whey protein emulsion-filled gels. *Food Research International*, *178*(January), 113987. https://doi.org/10.1016/j.foodres.2024.113987
- Grasberger, K., Vuholm Sunds, A., Hammershøj, M., & Corredig, M. (2024). Heat-induced lupin-whey protein emulsion-filled gels: Microstructural and rheological insights. *Lwt*, *201*(November 2023). https://doi.org/10.1016/j.lwt.2024.116269

Easily read papers:

- Short article on drop tensiometer technique for the general public and industry sent to Danish Centre for Food and Agriculture (DCA).
- Short article on project sent to Danish dairy board.

Student theses:

- PhD thesis. December 2023. Katherine Grasberger. Mastering structure design in model foods containing dairy proteins for flexitarian diets.
- Master thesis. June 2024. Anders Holste. Exploring for Potential Synergies between Faba Bean Proteins and Whey Proteins at the O/W Interface.

Oral presentations at scientific conferences, symposiums etc.:

- NIZO Plant Protein, Online, October 2020. Combining plant proteins and dairy proteins in a cream cheese model system shows synergies in structure and texture (Poster presentation).
- EFFOST. Lausanne, Switzerland. November 2021. Adsorption dynamics and viscoelastic properties of mixed plant-dairy protein interfaces formed through simultaneous and sequential adsorption (Oral presentation).
- Edible Soft Matter. Wageningen, The Netherlands. July 2022. Interfacial synergies of lupin and whey protein at the oil-water interface (Oral presentation).
- EFFOST. 2022. Food design challenges: Balancing sustainability, nutrition and circularity. (Keynote, Oral presentation).
- Dairy Australia webinar series. 2022. Hybrid dairy and plant proteins – opportunities for innovative foods (Oral presentation).
- Nordic Dairy congress. Malmo, Sweden. 2022. Dairy ingredients in food matrices dairy opportunities of to-morrow (Oral presentation).
- Nordic Rheology Conference. Aarhus, Denmark. April 2023. Colloidal States of Adsorbed Pea Proteins Deter-mined by Interfacial Shear Rheology (Poster presentation).
- International Whey Conference. Dublin, Ireland. September 2024. Going beyond dairy: Transforming plant-based emulsions with whey protein ingredients (Invited speaker, Oral presentation).

Oral presentations at meetings:

- Danish Dairy research foundation coordination meeting. September 2021. Proflex Progress Report: Mixed plant-dairy protein emulsions.
- Arla Foods Ingredients project updates. June 2022. ProFlex project status and future plans.
- Danish Dairy research foundation coordination meeting. September 2022. Project updates and future plans.
- Arla Foods Ingredients final report. April 2024. ProFlex Final report.
- Danish Dairy research foundation coordination meeting. June 2024. ProFlex Final report.

14. Contribution to master and PhD education

One PhD student and one master student was educated within the project

- PhD thesis. December 2023. Katherine Grasberger. Mastering structure design in model foods containing dairy proteins for flexitarian diets. Including guest research stay at ETH Zurich from January 2023 to April 2023.
- Masters thesis. June 2024. Anders Holste. Exploring for Potential Synergies between Faba Bean Proteins and Whey Proteins at the O/W Interface.

15. New contacts/projects

During the project, we have established a new scientific collaboration with Prof. Peter Fisher at ETH. Furthermore, we have strengthened our collaborations with Arla Food Ingredients, and we believe we will continue to pursue this area of research together in the future.

Sources

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