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Mario M. Martinez:

# STRING – Ekstrudering af mejeriproteiner til nye anisotrope fødevarer

## STRING – Extrusion of dairy proteins into novel anisotropic foods

Mejeribrugets
ForskningsFond

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# Final report

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

### 1. Title of the project

**Danish:** STRING: Ekstrudering af mejeriproteiner til nye anisotrope fødevarer

**English:** STRING: Extrusion of dairy proteins into novel anisotropic foods

### 2. Project manager


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### 4. Sources of funding


Milk Levy Fund and Arla Foods


### 5. Project period

**Project period with DDRF funding:** 08/2023 – 08/2025

### 6. Project summary

**Danish:**


Dette projekt havde til formål at designe og konstruere nye fødevarestrukturer med distinkte, justerbare teksturelle egenskaber og at udvikle fødevareprototyper, der spænder over en bred vifte af innovative teksturer baseret på termoplastiske matricer sammensat af mejeriproteiner og plantepolysaccharider. Parallelt søgte projektet at etablere en mekanistisk forståelse af molekylære, kolloide og mesoskalaprocesser, der styrer strukturdannelse under pilotskala-ekstrudering med høj fugtighed - et industrielt relevant, men utilstrækkeligt forstået forarbejdningsregime. Ved at integrere ekspertise inden for biopolymerbiokemi, termomekanisk fødevareforarbejdning og avanceret fødevarenanoteknologi

etablerede forskningen en flerskala-ramme, der forbinder molekylære interaktioner med makroskopisk tekstur og strukturel stabilitet, og dermed adresserer centrale videnshuller, der hindrer rationelt design og industriel oversættelse af næste generations teksturerede fødevaresystemer.

To komplementære studier dannede kernen i arbejdet. Det første studie undersøgte systematisk indflydelsen af stivelsesmolekylær arkitektur på fiberjustering, teksturudvikling og opbevaringsstabilitet i mælkeproteinkoncentrat (MPC)-baserede ekstrudater med høj fugtighed. Seks stivelser, der adskiller sig i amyloseindhold, kvældningsadfærd, gelatineringsegenskaber og tværbindingstæthed, blev evalueret. Strukturelle, teksturelle og mikro- til mesoskala-egenskaber blev vurderet både umiddelbart efter ekstrudering og efter 15 dages opbevaring, hvorved den indledende strukturdannelse og udvikling efter forarbejdning blev registreret. Majsstivelse med højt amyloseindhold fremmede overlegen fiberintegritet på grund af begrænset granulathævelse og forhøjet smelteviskositet, hvilket stabiliserede protein-polysaccharid-interaktioner under forskydning. Tilsvarende opretholdt tværbundet kartoffelstivelse sin bæreevne i proteinmatrixen, hvilket resulterede i forbedret strukturel stabilitet under opbevaring. Tapiokastivelse, der er karakteriseret ved mellemliggende hævelse og gunstig gelatineringsadfærd, fremmede kontinuerlig fasedannelse og effektiv stressoverførsel, hvilket understøttede kohærent fiberstrukturering.

Det andet studie fokuserede på at forbedre fiberdannelse og teksturel integritet gennem kontrolleret præhydrering af en defineret MPC-fraktion, der blev introduceret som flydende foder under ekstrudering. Ved at bruge formuleringer indeholdende nativ majsstivelse ændrede denne tilgang fundamentalt vandfordelingen under forarbejdning. Uden præhydrering dominerede stivelse vandoptagelsen, hvilket førte til overdreven hævelse og forringet proteinjustering. I modsætning hertil muliggjorde præhydrering en afbalanceret hydrering af både protein- og polysaccharidfaser, hvilket fremmede udviklingen af lamellær struktur under høj forskydning og resulterede i forbedret fiberjustering, tekstur og strukturel stabilitet.

Samlet set viser resultaterne, at robust fiberstrukturering i MPC-baserede ekstrudater med højt fugtindhold opstår fra den synergistiske optimering af stivelsesmolekylært design og hydreringsstrategi. Denne kombinerede tilgang giver en rationel vej til at skræddersy tekstur, anisotropi og stabilitet i mejeribaserede fiberholdige fødevaresystemer og tilbyder overførbare designprincipper for avancerede strukturerede fødevarer.

**English:**

This project aimed to design and engineer novel food structures with distinct, tunable textural properties and to develop food prototypes spanning a wide range of innovative textures based on thermoplastic matrices composed of dairy proteins and plant polysaccharides. In parallel, the project sought to establish mechanistic understanding of molecular, colloidal, and meso-scale processes governing structure formation during pilot-scale high-moisture extrusion—an industrially relevant yet insufficiently understood processing regime. By integrating expertise in biopolymer biochemistry, thermomechanical food processing, and advanced food nanotechnology, the research established a multi-scale framework linking molecular interactions to macroscopic texture and structural stability, thereby addressing key knowledge gaps that hinder the rational design and industrial translation of next-generation textured food systems.

Two complementary studies formed the core of the work. The first systematically investigated the influence of starch type on fiber alignment, texture development, and storage stability in milk protein concentrate (MPC)-based high-moisture extrudates. Six starches differing in amylose content, swelling behavior, gelatinization characteristics, and crosslink density were evaluated. Structural, textural, and micro- to meso-scale features were assessed both immediately after extrusion and after 15 days of storage, capturing initial structure formation and post-processing evolution. High-amylose maize starch promoted superior fiber integrity due to restricted granule swelling and elevated melt viscosity, which stabilized protein–starch interactions under shear. Similarly, crosslinked potato starch provided load-bearing capacity to the matrix, resulting in improved structural stability during storage. Tapioca starch, characterized by intermediate swelling, facilitated continuous phase formation and efficient stress transfer, supporting coherent fibrous structuring.

The second study focused on improving fiber formation and textural integrity through controlled pre-hydration of a defined MPC fraction introduced as a liquid feed during extrusion. Using formulations containing native maize starch, this approach fundamentally altered water partitioning during processing. Without pre-hydration, starch dominated

water uptake, leading to excessive swelling and impaired protein alignment. In contrast, pre-hydration enabled balanced hydration of both protein and polysaccharide phases, promoting lamellar structure development under high shear and resulting in enhanced fiber alignment, texture, and structural stability.
Collectively, the results demonstrate that robust fibrous structuring in MPC-based high-moisture extrudates emerges from the synergistic optimization of starch typology and hydration strategy. This combined approach provides a rational pathway to tailor texture, anisotropy, and stability in dairy-based fibrous food systems and offers transferable design principles for advanced structured foods.

## 7. Project aim

**Danish:**

Ekstrudering af partikelformige fødevareingredienser kan føre til ny supramolekylær fibrillær strukturdannelse ved at inducere nye intermolekylære interaktioner og tværbindinger mellem matrixbestanddelene. I protein-polysaccharid blandede systemer menes termodynamisk inkompatibilitet at være forløberen for anisotropidannelse under høj forskydning, som vi har set i nyere undersøgelser udført i vores gruppe. Det er derfor en hypotese, at mejeriproteiner, herunder proteinkoncentrater i flydende og pulverform, kan fungere som et stillads til dannelsen af flerfasestrukturer med høj anisotropi i tilstedeværelse af en hydrofil polymer dispergeret fase (f.eks. stivelse) under forskydningsspænding (høj forskydning efterfulgt af lav forskydningslaminering ved afkøling). Dette projekt har til formål at udvikle nye fødevarestrukturer med ekstremt forskellig teksturadfærd og fødevareprototyper med en bred vifte af nye teksturer fra en termoplastisk masse fremstillet af mejeriproteiner og plantepolysaccharider. Ydermere vil dette projekt give den meget tiltrængte forståelse af de molekylære og kolloide mekanismer, der er ansvarlige for strukturdannelse ved højfugtekstrudering i pilotanlægsskala. Den nuværende forskning kombinerer vores tidligere arbejde med biopolymer (polysaccharider og proteiner) biokemi, termomekanisk forarbejdning af plantebaserede fødevarer og avanceret fødevarenanoteknologi. Ingen af forskningsgrupperne, der er involveret i dette område, besidder fuldt ud værktøjerne og komplementariteten af molekylær forståelse end det team, der foreslås her. Dermed vil dette projekt skabe et hidtil uset omdrejningspunkt for viden i Danmark og udenfor.

**English:**

Extrusion of particulate food ingredients may lead to novel supramolecular fibrillary structure formation by inducing new intermolecular interactions and crosslinks between the matrix constituents. In protein-polysaccharide mixed systems, thermodynamic incompatibility is thought to be the precursor for anisotropy formation under high shear, as we have seen in recent studies conducted in our group. It is therefore hypothesized that dairy proteins, including protein concentrates in liquid and powder form, can act as a scaffold for the formation of multi-phase structures with high anisotropy in the presence of a hydrophilic polymeric dispersed phase (e.g., starch) under shear stress (high shear followed by low shear lamination upon cooling). This project aims to develop novel food structures with extremely different textural behaviors and food prototypes with a broad range of novel textures from a thermoplastic mass made from dairy proteins and plant polysaccharides. Furthermore, this project will provide the much-needed understanding about the molecular and colloidal mechanisms responsible for structure formation by high moisture extrusion at pilot plant scale. The current research combines our previous work on biopolymer (polysaccharides and proteins) biochemistry, thermomechanical processing of plant-based foods and advanced food nanotechnology. None of the research groups engaged in this space fully possess the tools and the complementarity of molecular understanding than the team proposed here. Thus, this project will create an unprecedented hub of knowledge in Denmark and beyond.

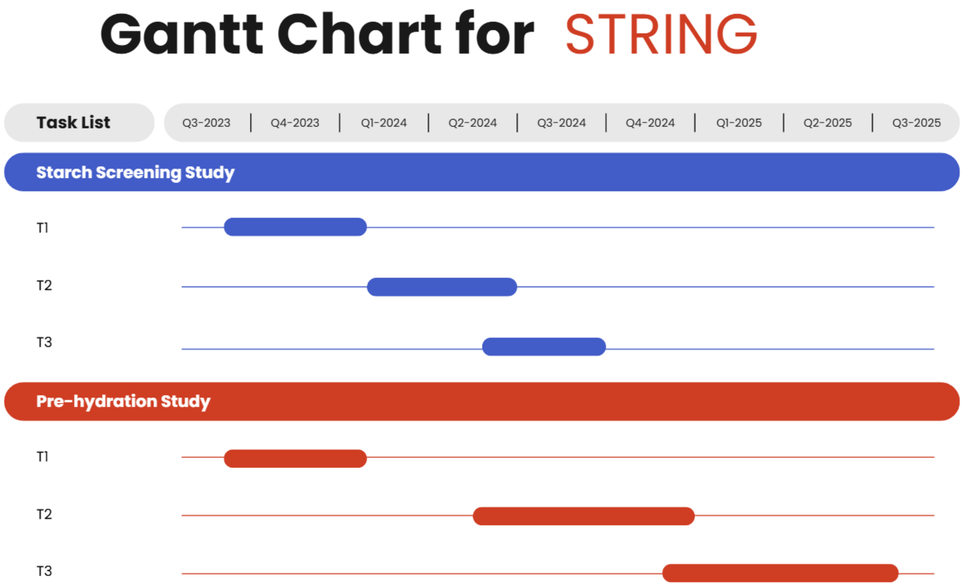
## 8. Background for the project

Over the past decade, the development of novel structured products has emerged as the most promising strategy to decrease meat consumption or result in novel textural attributes. Twin-screw extrusion, using low or high moisture, is the most used commercial technique to transform materials into fibrous products for several reasons. Firstly, the thermomechanical stresses generated in the screw-barrel assembly induce structural changes on the material, which is then forced to flow through the die, where the final structure and shape of the products is defined [Bouvier, & Campanella, 2014; *Extrusion processing technology: Food and non-food biomaterials*]. Extrusion is hence one of a kind to generate tensile and shear stresses in a continuous operation. Secondly, extrusion clearly meets process-intensifying requirements from technical and economic standpoints [Bouvier, & Campanella, 2014]. For example, twin-screw extrusion has the potential to replace the typical cooker-stretcher mainly used for mozzarella manufacture, since it is more flexible in terms of texture development. This is due to the broad range of thermal and mechanical energy inputs and efficient mixing, while minimizing loss [Feng, et al., 2021; *Journal of Food Engineering, 309*]. The independent modification of extrusion parameters offers flexibility and enables to structure foods with a diversity in properties, provided that the polymers behave in such a manner that result in structure. In high moisture extrusion (HME) (moisture > 40%) of protein and polysaccharide mixtures, starch will gelatinize (fully or partially), and the hydrated protein will denature, reaching a state called melt which is fundamental to obtain an irreversible dense fibrous structure after cooling. Phase separation is generally considered to be a first step towards fiber formation, which is mainly due to protein-polysaccharide incompatibility [Tolstoguzov, 2006, *Biotechnol Adv, 24*; Corredig, Sharafbafi, and Kristo, *Food Hydrocolloids, 25*].

In our previous collaboration with Arla in the framework of a MSc project, we investigated the behavior of bovine milk protein concentrates (in powder form) with pea starch during high moisture extrusion using a laboratory-scale mini-twin-screw extruder [Skov, Suwal, Corredig, Martinez, New fibrous protein structures formed with high moisture extrusion of milk protein concentrates. *Food Hydrocolloids, 109645*]. With this study we found that extrusion resulted in successful protein fibrillation, and we established the effect of starch and water concentrations and temperature profiles on visual and instrumental texture, and water mobility. We believe that these systems could have potentially benefited from a longer cooling die that supplies greater cooling capacity, which can be studied in the industrial scale extruder.

Finally, we also hypothesized that milk protein concentrates in liquid form could be important building blocks for the design of future hybrid products with unique texture and less resources (i.e. less steps in terms of water removal). Alongside a potential competitive advantage in terms of functionality, the direct processing of liquid concentrates (fed as liquid stream in the screw-barrel assembly) would by-pass the processing steps for water removal and, therefore, result in important energy savings. This is a very novel approach that requires strong complementary expertise in polysaccharide and protein biochemistry and physics, unique to the team in this project. Furthermore, none of the research groups engaged in this space fully possess the tools and the complementarity of molecular understanding than the team proposed here. Thus, this project will create an unprecedented hub of knowledge in Denmark and beyond within Arla's priority areas "new ways to approach sustainability", "physical functionality of milk-based ingredients" and "novel methodologies to investigate milk ingredients".

## 9. Sub-activities in the entire project period




The planned activities for the STRING project are organized into two major study phases—**Starch Screening Study** and **Pre-hydration Study**—each consisting of three sequential tasks (T1 to T3). The timeline spans from **Q3-2023 through Q3-2025**.

__**Starch Screening Study (Q3 2023 – Q3 2025)**__

This study is broken down into three core sub-activities:

**T1 (Q3 2023 – Q1 2024):** Initial screening of various starches focusing on physicochemical and functional properties, such as amylose content, swelling capacity, and gelatinization behavior. It also included formulation, sample preparation, and preliminary rheological assessments.

**T2 (Q1 2024 – Q2 2024):** Pilot-scale extrusion trials using selected starches from T1, evaluating their performance under controlled high-moisture extrusion conditions. This phase emphasizes structural analysis (fiber formation, anisotropy) and water partitioning behavior.

**T3 (Q2 2024 – Q3 2024):** Final refinement phase involving advanced structural characterization, including microscopy (confocal), mechanical testing, and stability assessments.

__**Pre-hydration Study (Q3 2023 – Q3 2025)**__

This study was initiated in parallel and focuses on the strategic pre-hydration of milk protein concentrate (MPC). It includes:

**T1 (Q3 2023 – Q1 2024):** Method development for MPC pre-hydration and integration into extrusion workflow. Includes optimization of hydration levels, solubility assessments, and pre-processing compatibility tests.

**T2 (Q1 2025 – Q2 2025):** Full-scale extrusion trials incorporating pre-hydrated protein, with comparative evaluation against non-hydrated controls. Outcomes target structural development, lamellar formation, and textural improvements.

**T3 (Q2 2025 – Q3 2025):** Final validation phase, likely involving advanced structural and mechanical analysis (e.g., lamella continuity, textural profiling).

## 10. Deviations

**Scientific deviations**

From a scientific and technical perspective, the only notable deviations from the original experimental framework occurred during the pilot-scale production phase using the food-grade twin-screw extruder. These deviations were primarily driven by two factors: first, the scope of the project was expanded mid-way to incorporate a new experimental section focused on the pre-hydration of milk protein concentrate (MPC), which necessitated additional trials, processing conditions, and analytical evaluations. This addition significantly increased the complexity and duration of the extrusion trials.

Second, there were logistical constraints related to the availability of critical raw materials—most notably the limited supply of MPC, which impacted the continuity and scale of pilot runs. As a result, it was not possible to carry out the planned food-grade extrusion under conditions suitable for downstream sensory evaluation.

Last, we would like to mention that the Postdoc working on this project got a position at Novo Nordisk and left Aarhus University 3 months before the project ended.

Due to the inability to produce extrudates that met food safety and regulatory standards and the time constrains mentioned above, the originally proposed sensory trials—intended to assess the organoleptic properties of the newly developed MPC-based hybrid structured products—could not be executed. Consequently, while the structural and rheological characterization of extrudates proceeded under lab-scale or semi-technical conditions, the validation of product quality from a consumer acceptability perspective remains a subject for future investigation once food-grade processing can be resumed.

## 11. Project results

The behavior of bovine milk protein concentrate investigated under different extrusion conditions in mixtures with different starches varying in colloidal behavior (e.g., potato starch, maize starch) and at different barrel moistures. Extrusion trials conducted using lab scale extruders - equipped with co-rotating, intermeshing twin-screws and a length to diameter (L/D) of 40, a cooling section, and several ports for the cross-feeding of solid and liquid streams at different parts of the screw-barrel assembly. This project is divided in the following tasks (T) that were performed in a 24-month period;

### D1: Characterization of milk proteins and starches (T1)

Proximate composition of spray-dried milk concentrate (Fromaquick® Milk protein Concentrate, MPC) was determined as 6.44% moisture, 85% (w/w d.b.) protein, 2% (w/w d.b.) total fat, and 2.5% (w/w d.b.) lactose. MPC was supplied by Fayrefield Foodtec A/S (Denmark). On the other hand, structural and functional properties of the selected starches were either investigated within the scope of the project or compiled from the previous works of our research group (**Table 1**). The methods/techniques used to determine the defined properties might be summarized as follows: High-Performance Anion Exchange Chromatography (HPAEC) and Size-Exclusion Chromatography (SEC) to detect amylose and amylopectin content; Scanning Electron Microscopy (SEM) to determine granule morphology; Laser Diffraction Particle Size Analyzer for granule size distribution; Differential Scanning Calorimetry (DSC) for thermal properties; Gravimetric method (swelling in water at different temperatures) and centrifugation to understand swelling power and solubility; Rapid Visco Analyzer (RVA) and Brookfield viscometer for pasting and viscosity measurements.

**Table 1. Structural and functional properties of starches**

| Strach type | Amylose content (%) | Amylopectin content (%) | Granule morphology | Swelling power | Gelatinization Temperature (°C) | Viscosity behavior | Viscosity (cP, 95 °C) | Reference |
|---|---|---|---|---|---|---|---|---|
| Waxy maize | <5 | >95 | Small polygonal, smooth | High | 63–72 | High peak viscosity, unstable | 4500 | Beech et al., 2022; Jia et al., 2023 |
| Maize | 20-30 | 70-80 | Round/polyhedral | Moderate | 65–75 | Moderate, some breakdown | 3200 | Beech et al., 2022; Martínez & Gómez, 2017 |
| High-amylose maize | 50-70 | 30-50 | Small, irregular, compact | Low | >100 | Low peak, stable | 900 | Beech et al., 2022; Luo et al., 2023 |
| Tapioca | 1-20 | 80-83 | Large, round to oval | Very high | 68–78 | High, cohesive paste | 5200 | Saiprabhath et al., 2026; Jia et al., 2023 |
| Potato | 20-25 | 75-80 | Large, irregular, oval | Very high | 58–66 | Very high, unstable paste | 6000 | Ubaid et al., 2026; Compart & Fettke, 2025 |
| Crosslinked potato | 20-25 | 75-80 | Slightly deformed granules (due to crosslinking) | Reduced (due to crosslinking) | Higher than native | Improved stability under heat and shear | 2700 | Roman et al., 2020; Punia Bangar et al., 2024 |

## D2. Anisotropic structuring by high moisture extrusion (T2)

Both the starch screening and pre-hydration studies were conducted with a 400 rpm screw speed, 20 g/min total output, 7.78 g/min mass of solids and 12.2 g/min water flow rate for 63% barrel moisture (BM) while 6.73 g/min mass of solids and 13.3 g/min water flow rate for 68% BM. Temperature profile throughout the barrel from inlet towards the die heater was 45°C, 60°C, 75°C, 80°C, 100°C, 90°C, 75°C, and 40°C, respectively (Nasrollahzadeh, et al., 2022; Skov, et al., 2024). The detailed information regarding sample composition, processing parameters and final product contents are provided in Table 2.

**Table 2. Processing parameters and final product properties**

| | Sample name | Pre-hydrated MPC in water feed (g/100 mL) | Starch Type | Starch content in the dry feed (g/min) | Barrel moisture (%) | Final protein content (%) | Final starch content (%) |
|---|---|---|---|---|---|---|---|
| Starch Screening Study | Control | - | - | 0 | 63 | 33.0 | - |
| | Waxy | - | Waxy maize | 1.1 | 63 | 28.4 | 5.4 |
| | Maize | - | Maize | 1.1 | 63 | 28.4 | 5.4 |
| | High-amylose | - | High amylose maize | 1.1 | 63 | 28.4 | 5.4 |
| | Tapioca | - | Tapioca | 1.1 | 63 | 28.4 | 5.4 |
| | Potato | - | Potato | 1.1 | 63 | 28.4 | 5.4 |
| | Crosslinked Potato | - | Crosslinked potato | 1.1 | 63 | 28.4 | 5.4 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Pre-hydration Study | Control-1 | 0 | - | 0 | 63 | 33.0 | - |
| | MPC | 0 | Maize | 1.1 | 63 | 28.4 | 5.4 |
| | DisMPC | 15 | Maize | 1.1 | 63 | 28.4 | 5.4 |
| | Control-2 | 0 | 0 | 0 | 68 | 28.5 | - |
| | HM-MPC | 0 | Maize | 1.1 | 68 | 24.5 | 4.7 |
| | HM-DisMPC | 15 | Maize | 1.1 | 68 | 24.5 | 4.7 |
| | HM-DisMPC-HP | 15 | Maize | 0.7 | 68 | 34.1 | 3.3 |

Sample names of the starch screening study refer to the MPC extrudates with the declared starch type. Control refers to only MPC extrudate with no starch addition. Sample names of the pre-hydration study refer to Control-1 and -2 only MPC extrudates with no starch addition with a barrel moisture of 63 and 68%, respectively. The all-other samples in this study contain starch. DisMPC refers to containing dissolved MPC; HM refers to high barrel moisture; HP refers to high protein.

The hand-broken macro images of produced samples for starch screening study (for day-1 and day-15) and of pre-hydration study can be seen in Fig. 1 and Fig. 2, respectively.

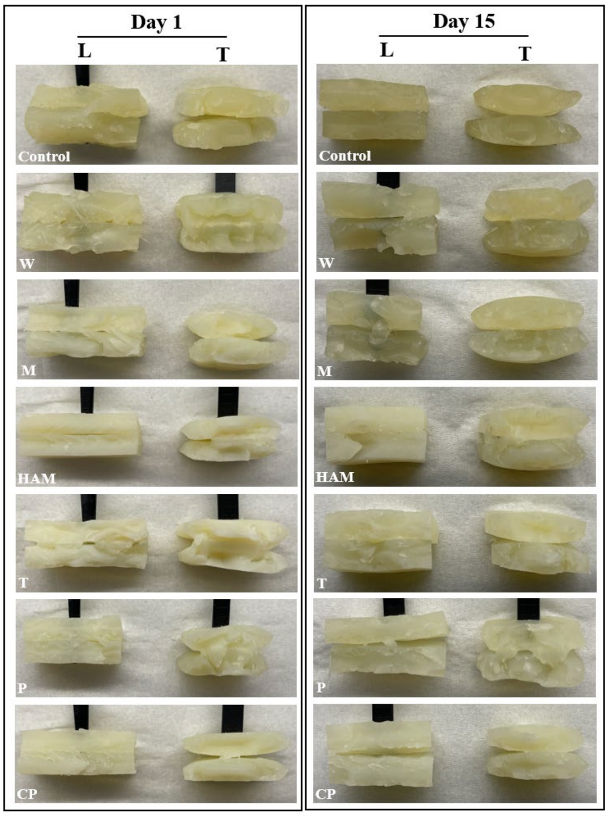



**Figure 1**. Hand-broken macro images of starch screening study samples at day 1 and day 15.

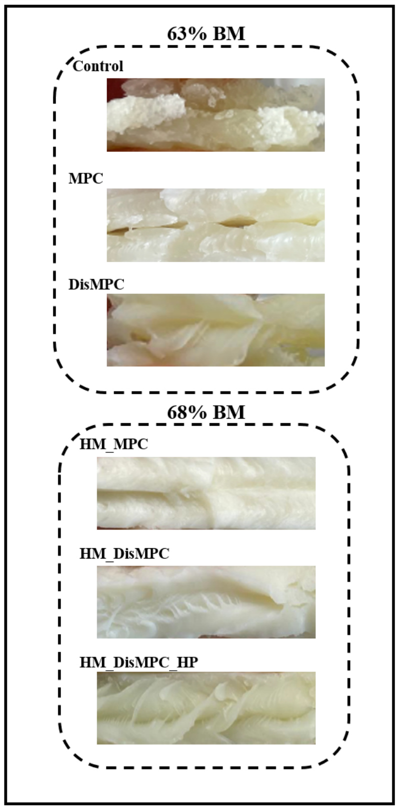



**Figure 2**. Hand-broken macro images of pre-hydration study samples.

In this task, extensive range of anisotropy formations were able to be created as hypothesized either incorporating starches with distinctive properties (Fig. 1) or pre-dissolving only 15% of the total needed MPC prior to extrusion (Fig. 2). It should be emphasized that pre-dissolving attempt made it possible to produce a final extruded sample with a relatively higher protein content (34.1% instead of 28.4%), despite being produced with higher BM ratio (68% instead of 63%). By closely mimicking milk, the 15% dissolved MPC serves as a proof of concept that milk itself can be directly processed into novel structured food prototypes within remarkably short processing times via high moisture extrusion.

### D3. Protein-polysaccharide interactions and molecular conformation changes and characterization of anisotropic structured products (T2 & T3)

In this section, protein–polysaccharide interactions and molecular conformational changes were investigated using confocal laser scattering microscopy (Fig. 3 and Fig. 4), while water partitioning was assessed by low-field NMR and amylopectin retrogradation by differential scanning calorimetry (Fig. 5 and Fig. 6). The physical structure of the final extrudates was further characterized by measuring cutting force using Warner–Bratzler shear force analysis and surface whiteness (L*) using hyperspectral imaging.

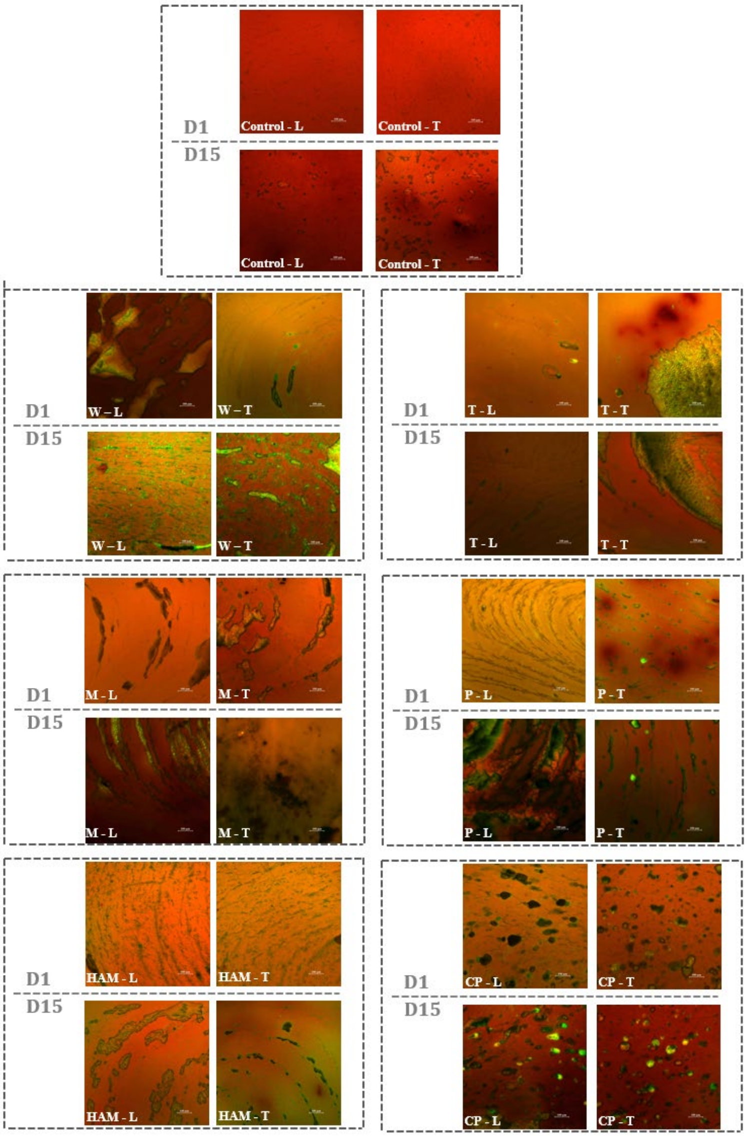



**Figure 3**. CLSM images of clean-cut extruded samples from starch screening study longitudinally (L) in the direction of flow and transversal (T) to the direction of flow.

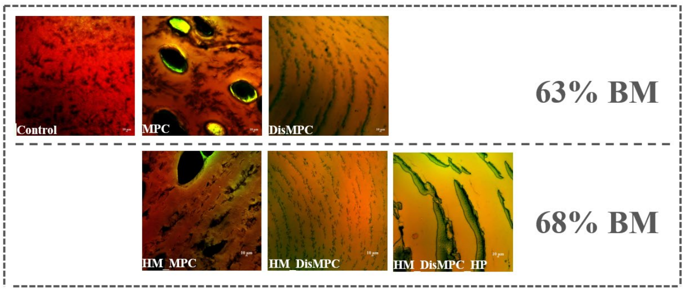



**Figure 4**. CLSM images of clean-cut extruded samples from pre-hydration study longitudinally (L) in the direction of flow and transversal (T) to the direction of flow.

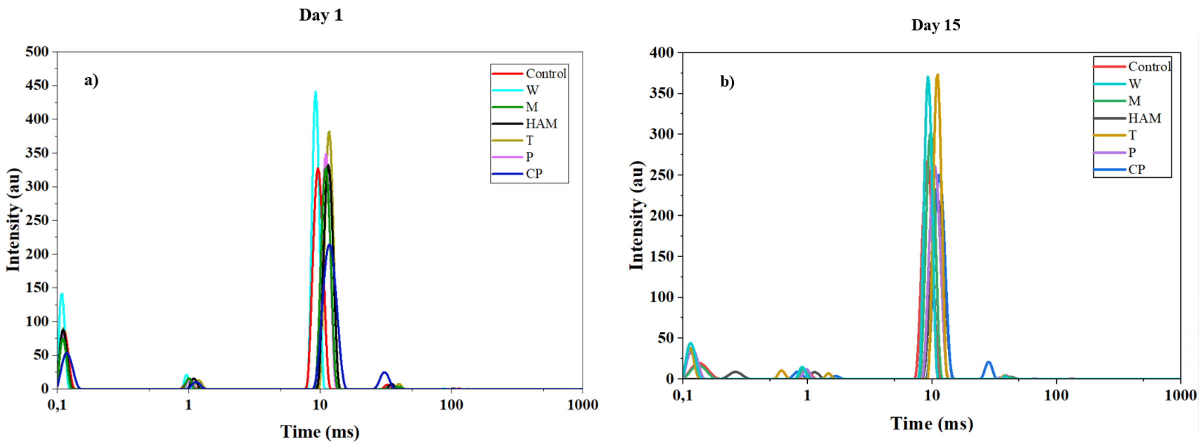



**Figure 5.** Low-field NMR $T_2$ relaxation distributions of extruded starch screening study samples on Day 1 (a), and Day 15 (b) showing starch-dependent differences in water mobility.

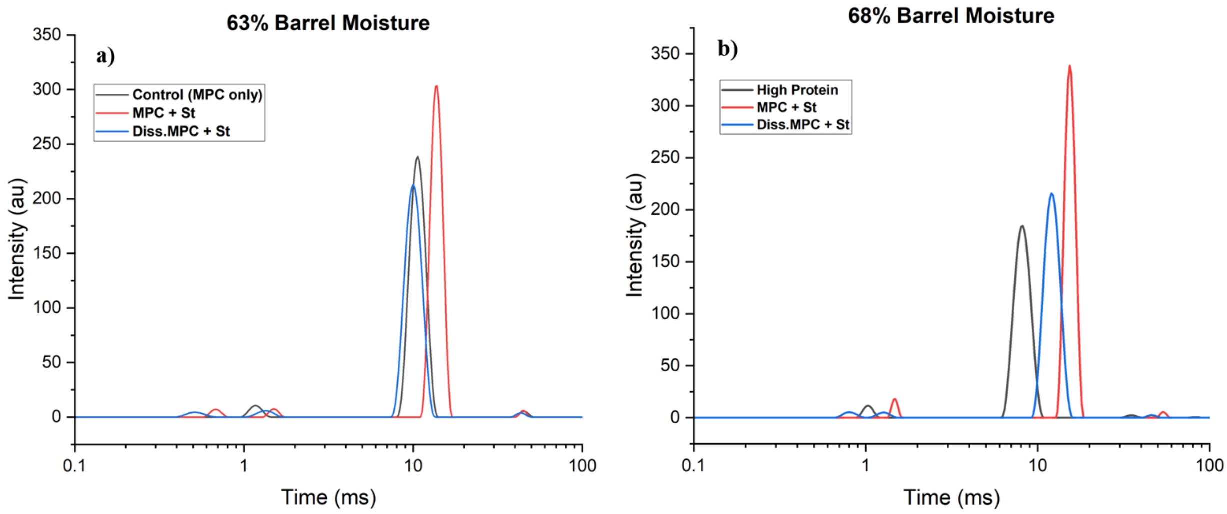



**Figure 6.** Low-field NMR $T_2$ relaxation distributions of extruded pre-hydration study samples processed at 63% BM (a), and 68% BM (b).

Based on the provided results, starches differing in amylose content, granular swelling capacity, gelatinization behavior, and crosslink density were evaluated to elucidate their impact on water partitioning, phase behavior, and anisotropic fiber formation (Roman et al., 2020). High-amylose maize starch and crosslinked potato starch, characterized by restricted swelling and higher resistance to shear-induced disintegration, promoted stable protein–polysaccharide networks and superior structural integrity during storage. In contrast, highly swelling starches with rapid gelatinization tended to dominate water uptake, disrupting protein alignment and reducing fiber continuity. These findings demonstrate that controlled starch swelling and melt rheology, rather than starch presence per se, are critical determinants of fibrous structure development (Kang et al., 2026). The formation of lamella structures can be tracked with microscopy images as well as with some macro images.

Pre-hydration approach fundamentally altered competitive water absorption during processing. Without pre-hydration, starch preferentially absorbed available water, leading to excessive granule swelling and gelatinization that hindered protein fibrillation. Pre-hydration enabled milk proteins to reach a hydrated, deformation-ready state prior to shear exposure, allowing both protein and starch phases to participate in structure formation (Zhang et al, 2018). As a result, well-defined lamellar architectures, enhanced fiber alignment, and improved textural stability were obtained under high-shear conditions. Similarly, these facts can be confirmed by the macro and microscopy images.

On the other hand, the results from DSC, Warner-Bratzler shear force (WBSF), and whiteness measurements did not yield meaningful distinctions in either study regarding the contribution of starch type to anisotropy formation or the impact of pre-hydrated milk protein on lamellar structuring. Consequently, these data were excluded from the report, as they did not contribute to the critical discussion. Overall, the project establishes starch selection based on molecular functionality and protein pre-hydration as key levers for tailoring texture, water mobility, and stability in hybrid extrusion systems. These insights provide scalable strategies for industrial application, unlock new valorization routes for liquid dairy streams, and open future research directions in liquid-based protein processing, advanced extrusion design, and predictive structure–function modeling for sustainable hybrid foods.

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

This project directly addressed circularity and the reduction of resource-intensive foods and ingredients, aligning closely with the Danish Dairy Research Foundation's Strategy 2026. By advancing the fundamental understanding of the molecular and colloidal properties of milk constituents and their interactions with plant-based polysaccharides such as starch during processing, storage, and end-use, the project generated scientifically robust knowledge essential for the rational design of next-generation food systems.

From a scientific perspective, the results provided new insight into how dairy proteins behave as functional building blocks within hybrid matrices under thermomechanical stress, bridging molecular-scale interactions with macroscopic structure and texture development. These findings open new research avenues in hybrid food structuring, including the targeted modulation of protein–starch interactions, phase behavior, and water mobility to tailor texture and stability in complex food systems.

From a societal perspective, the project supports the transition toward more sustainable dietary patterns by enabling the development of hybrid and alternative food products with reduced environmental footprints and high sensory appeal. By facilitating partial substitution of meat through dairy–plant hybrid structures, the outcomes contribute to improved resource efficiency while maintaining consumer acceptance through desirable texture and eating quality.

From a dairy industry perspective, the project unlocked new value streams by demonstrating the feasibility of utilizing dairy by-products, including liquid side streams, as functional ingredients for novel food applications. This approach not only enhances ingredient circularity but also reduces energy-intensive processing steps, thereby improving both economic and environmental performance. The ability to generate novel textures with meat-like or entirely new sensory characteristics positions Arla Foods at the forefront of innovation in hybrid food development.

Importantly, the results pave the way for new research directions, including scale-up studies at industrial extrusion facilities, the exploration of enzymatic or physicochemical pre-treatments to further tailor functionality, and the integration of digital and data-driven approaches to predict structure–function relationships in hybrid matrices. Collectively, this project establishes a strong foundation for future interdisciplinary research and reinforces Denmark's leadership in sustainable dairy innovation.

## 13. Communication and knowledge sharing about the project

**Papers in international journals:** A manuscript is being prepared for submission to the journal "Food Structure" published by Elsevier.

**Easily read papers:** NA

**Student theses:** NA

**Oral presentations at scientific conferences, symposiums etc.:** NA

**Oral presentations at meetings:** NA

**Other:** NA

## 14. Contribution to master and PhD education

This project did not contribute to any PhD study, but it did contribute to the hosting of 3 MSc students from France.

## 15. New contacts/projects

This project has stablished new lines for food structuring using dairy products that resulted in the preparation of several proposals that will be submitted to different instruments, including Innovation Fund Denmark.

## 16. References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