Structural Development of Oat-Based Yoghurt

The Role of Ultrasound, Protein Blending, and Calcium

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Title Structural Development of Oat-Based Yoghurt: The Role of Ultrasound, Protein Blending, and Calcium

Strukturutveckling hos havrebaserad yoghurt: effekter av ultraljud, proteinblandning och kalcium

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Abstract

This study evaluated the structural development of an oat-based yoghurt analogue utilising an oat protein concentrate (OPC: PrOatein, 55% protein). The primary objective was to investigate how combining OPC with pea protein isolate (PPI: Pisane, 83.6% protein), adjusting ionic strength via $CaCl_2$ addition and applying ultrasonication could modulate hydration, interfacial and rheological properties of the system to achieve a desirable protein gel network suitable for yoghurt texture.

Protein dispersions were formulated with varying ratios of OPC and PPI (100:0, 90:10, 75:25) or fortified with 30 mM and 100 mM $CaCl_2$. First, physicochemical attributes with and without ultrasound were analysed including water solubility index (WSI), water holding capacity (WHC), foaming capacity (FC) and emulsifying capacity (EC). The ultrasound effect was evaluated via light microscopy. Second, sonicated milk analogues were created and pasteurised, thereafter inoculated with a commercial yoghurt starter culture and fermented at 40°C. The milk and yoghurt analogues were evaluated rheologically (viscosity and flow curves). The network was analysed via confocal laser scanning microscopy (CLSM).

Light microscopy visually confirmed particle size reduction by sonication, which transformed large, dense aggregates into a finer disperse matrix and increase in WSI and improved interfacial properties. EC was improved with sonication and higher inclusion of PPI.

All formulations exhibited shear thinning (pseudoplastic) behaviour. At neutral pH PPI decreased the viscosity, possibly due to electrostatic repulsion. However, after fermentation viscosity increased, 75:25 (OPC:PPI) blend was the only sample that transitioned from a liquid into an acid induced gel. The sample showed highest shear stress and a distinct yield stress of approximately 24 Pa. The calcium fortified samples induced pre-aggregation (salting out), which hindered network formation and resulted in lower resistance than the non-fortified oat milk and yoghurt. CLSM supported the rheological findings by revealing isolated aggregates in the calcium systems, whereas the 75:25 sample displayed a highly dense, continuous protein network.

These findings demonstrate that the OPC ingredient with gelatinised starch alone was insufficient to form a continuous, yoghurt structure under tested conditions. Instead, including PPI resulted in protein gelation near isoelectric point. Thus, incorporating a threshold concentration of at least 25% PPI in relation to OPC, combined with ultrasonication represents a processing strategy to build structural integrity and enhance physicochemical properties of the OPC.

Keywords: plant-based yoghurt, oat protein concentrate, pea protein isolate, ultrasonication, acid-induced gelation, rheology, microstructure

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Abbreviations

	General abbreviations
CLSM	Confocal laser scanning microscopy
EC	Emulsifying capacity
FC	Foaming capacity
FS	Foaming stability
LM	Light microscopy
OPC	Oat protein concentrate
OPI	Oat protein isolate
pI	Isoelectric point
PPI	Pea protein isolate
WHC	Water holding capacity
WSI	Water solubility index

	Sample abbreviations
Ca30	100% OPC + 30 mM $CaCl_2$
Ca100	100% OPC + 100 mM $CaCl_2$
Oat	100% OPC
Pea10	90% OPC + 10% PPI
Pea25	75% OPC + 25% PPI

1. Introduction

The requirement for complementary protein sources in addition to traditional dairy and meat is increasingly important. Recent disruptions in global food supply chains have highlighted the necessity of a resilient national food system, which can be achieved through the diversification of raw materials and greater self-sufficiency in agricultural production (Mešić et al. 2024).

Incorporating nitrogen-fixing legumes into cropping systems is one effective strategy to reduce dependence on external inputs such as synthetic fertilisers. For example, Tamiru et al. (2023) demonstrated that intercropping oat and field pea can maintain or increase yields while simultaneously reducing environmental impact. This agricultural synergy supports the co-utilisation of oat and pea, aligning with recent domestic industrial developments such as the investment of Lantmännen in a pea protein isolate (PPI) factory in Sweden (Lantmännen 2024).

Oats are of particular interest due to their content of beta-glucan, which is widely associated with beneficial effects on cholesterol and glycaemic response, as well as their suitability for individuals with celiac disease (Lazaridou & Biliaderis 2007). Complementary, pea protein provides a highly functional, non-allergenic alternative to imported soy (Burger & Zhang 2019).

Consequently, there is a clear need to develop novel food products that utilise crops beyond traditional applications. The focus of this project was to evaluate the structural development of an oat-based yoghurt analogue. In a previous study by Wang et al. (2023), pure oat yoghurt was found to be too thin, whereas soy yoghurt was thicker than traditional dairy options. Thus, there is considerable potential to combine oat with a pulse source to achieve a desirable, dairy-like texture. In this study, PPI was utilised instead of soy to produce a Swedish, plant-based yoghurt complement with a complete amino acid profile.

1.1 Aim

The aim of this work was to evaluate the structural development of oat-based yoghurt by combining protein sources through the addition of PPI, adjusting ionic strength via $CaCl_2$ fortification, and applying ultrasonication processing.

2. Theoretical Background

2.1 Composition of the Oat Kernel

Oats, *Avena sativa*, are dehulled before industrial use, separating the outer hull from the edible kernel (groat), which consists of the bran, endosperm and germ. The composition of the oat kernel differs from other common cereals, such as wheat and barley. Notably, oats lack the gluten forming properties required for traditional baking applications. However, they offer a distinct nutritional profile characterised by a prominent starch fraction, a better-balanced amino acid composition, a high lipid concentration and a substantial reserve of health-beneficial soluble dietary fibre (Butt et al. 2008).

2.1.1 Starch Fractions

Starch is the primary energy reserve of the oat grain and its most abundant component, accounting for approximately 50% of the total dry weight of the kernel. Oat starch granules are distinctively small compared to those of other cereals, typically ranging between 2-12 μm in diameter and show a structural tendency to form aggregate clusters (Punia et al. 2020).

Traits unique to oat starch include relatively short amylose chain length and high relative crystallinity (Punia et al. 2020). From a functional standpoint, these structural properties directly dictate gelatinisation behaviour during thermal processing, which subsequently defines the final viscosity and overall structure matrix of oat-based food products. Understanding these properties is vital when formulating applications with unpurified ingredients like oat protein concentrate (OPC), where starch remains a major functional, viscosity-building constituent.

2.1.2 Protein Molecular Structure

Following starch, proteins represent the second most dominant macro-constituent in the oat groat, with concentrations typically 12-15% depending on genotype and growth environments (Janssen et al. 2025). According to the historic Osborne classification scheme, proteins are divided into four distinct fractions based on their solubility profiles: water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamins, and dilute acid/base-soluble glutelins (Kumar et al. 2021)

Unique among cereal grains, the dominant protein fraction in oats consists of storage globulins which comprise 70-80% of the total protein content (Janssen et al. 2025), whereas prolamins (termed avenins for oat) represent a minor factor,

which is the most prevalent fraction in other cereals. This biological distinction provides oats with a superior nutritional profile compared to wheat and barley, as oat globulins contain higher ratio of lysine and histidine, alongside rich reserves of aspartic acid. The water-soluble albumin fraction is similarly rich in lysine and alanine (Kumar et al. 2021).

Structurally, oat globulins are primarily composed of high-molecular-weight 12S hexamers (320 kDa), with minor 7S and 3S configurations present. Each 12S monomer consists of an acidic subunit and a basic subunit linked together via a covalent disulphide bond, forming tight trimers that associate into the final hexameric (Janssen et al. 2025). Oat globulin has higher heat stability than most plant proteins and denatures at approximately 110°C (Wang et al. 2023).

2.1.3 Non-Starch Polysaccharides

Oats consist of non-starch polysaccharides, serving as dietary fibres which are indigestible and provide health benefits for humans (Xu 2012). There are insoluble dietary fibres in oats including cellulose, xylans and lignin (Frølich & Nyman 1988). The most prevalent soluble dietary fibre in oats is β-glucan, located primarily in the endospermic cell walls. It has hydrocolloid properties that provides a viscous effect both within food systems as well as in the gut. This high viscosity is dependent on high molecular weight, which is associated with blood serum cholesterol reduction and regulated blood glucose responses (Lazaridou & Biliaderis 2007).

In aqueous solutions, the concentration and molecular weight of β-glucan affect rheological manners, specifically its capacity to increase viscosity and form weak gel structures (Butt et al. 2008). This hydrocolloidal thickness is used in industry to modify texture and improve mouthfeel in low-fat products (Lazaridou & Biliaderis 2007).

In a complex ingredient like OPC, the presence of dietary fibres alters the functional properties of the protein by imposing steric hindrance and competitive water binding.

2.1.4 Unsaturated Lipids

The lipid content of oats, average 7% (Smulders et al. 2018a), is substantially higher than other cereals. Additionally, oat lipids are distributed throughout the kernel in contrast to most cereals where they are concentrated within the germ (Butt et al. 2008). The oat lipids are highly unsaturated, dominated by oleic acid and linoleic acid, which are associated with heart health and maintaining healthy blood fat levels

(Smulders et al. 2018a). However, it makes them susceptible to oxidative and enzymatic rancidity.

To mitigate this, oats are typically hydrothermally processed after dehulling to denature and inactive endogenous lipase enzymes (Butt et al. 2008). This processing step is critical to prevent lipid hydrolysis, which would cause off-flavours and shelf-life degradation (Smulders et al. 2018b).

Lipids in an OPC affect the protein functionality by influencing protein adsorption kinetics and thereby altering macro-properties like emulsification and foaming.

2.2 Pea Protein Properties

Yellow peas, *Pisum sativum*, are rich in protein, with 20-30% of their total mass, and are rich in the essential amino acid lysine. Like oats, the protein profile of pea is dominated by storage protein globulins (65-80%), with metabolically active albumins comprising the remaining. The globulins are divided into two fractions: legumin 11S and vicilin 7S (Burger & Zhang 2019) and some amount of convicilin (Lam et al. 2018).

Given the similarities between soy and pea proteins (Klost et al. 2020a), soy (and pea) 7S globulin (trimeric, 150-200 kDa) and 11S globulin (hexamer, 300-400kDa) are related to gelling properties, however oat globulins consist primarily of 12S globulin (hexamer, 320 kDa).

2.3 Protein Concentrates and Isolates

The extraction method for plant protein separation directly dictates the purity, structural integrity, and technical performance of the final ingredient. High-purity isolates are typically manufactured using wet extraction, such as alkaline dissolution followed by isoelectric precipitation or salt extraction. Because these intense processes remove most non-protein components that would otherwise physically interfere with protein-protein alignment, isolates exhibit higher initial solubility, foaming capacity, and interfacial properties (Spaen & Silva 2021).

However, wet extraction is resource-intensive and can affect native protein conformation due to chemical adjustment. In contrast, milder techniques like dry fractionation and milling are utilised to produce protein concentrates. While dry fractionation is a sustainable, low-energy requiring method that preserves the native state of the protein molecules, the resulting concentrates possess a lower protein purity and retain quantities of starches, fibres, and unsaturated fats (Spaen & Silva 2021).

2.4 Physicochemical Properties of Proteins

2.4.1 Solubility

Solubility is the primary thermodynamic property that governs all secondary functionalities of a protein in an aqueous system. A protein must be effectively dispersed or dissolved to form a foam or stabilise an emulsion. Insoluble proteins create precipitates that impair the technological properties. However, if the protein reaches its saturation limit, the protein will start to precipitate. Protein solubility is dictated by a balance between electrostatic and hydrophobic interactions, which are modified by environmental pH and ionic strength (Grossmann & McClements 2023).

At a protein's isoelectric point (pI), the net electrical charge of the molecule is zero, minimising electrostatic repulsion and causing proteins to aggregate via surface hydrophobicity, causing low solubility. At pH values further away from the pI of a protein, there will be repulsive interactions due to positive or negative charges, enhancing solvent interaction by keeping the molecules separated (Grossmann & McClements 2023).

Regarding ionic strength, the solubility of a protein might be increased by low salt concentration by the ionic interaction with charged group on protein surface, this leads to higher water interaction thus better solubility. This is known as salting-in phenomena. Conversely, at very high concentrations of salt, it will compete with the charged amino acid over free water for salt to stay dissolved. Additionally, the unsolved amino acids will precipitate. This results in poorer protein solubility (Li & Xiong 2021).

Oat proteins generally exhibit lower baseline water solubility compared to pulse protein isolates across neutral and acidic regions. This limitation is partly due to structural surface of oat globulins, which contain extensive glutamine-rich regions, thereby making it less hydrophilic than other plant globulins (Spaen & Silva 2021). Furthermore, the industrial hydrothermal kilning required to prevent oat rancidity induces induce protein denaturation (primarily albumin and prolamin fraction) and aggregation, thus reducing the native protein solubility from 74.6% to 35.7% (Runyon et al. 2015).

Within the individual fractions, oat albumins and glutelins exhibit the highest WHC (2.4mL/g and 1.9mL/g, respectively), whereas native globulins and prolamins absorb notably less moisture (0.8 mL/g) (Mel & Malalgoda 2022a). WHC is fundamentally governed by this solubility and particle size distribution.

Mechanically reducing particle size via processing strategies like ultrasonication expands the accessible surface area, providing more physical binding sites to entrap water and modify hydration properties (Dhakal et al. 2025).

2.4.2 Foaming Properties

A foam is a thermodynamically unstable colloidal dispersion consisting of gas bubbles suspended within a continuous liquid or solid matrix. Proteins retard foam collapse by acting as surfactants that lower interfacial tension and build a thin, protective viscoelastic film around the trapped air bubbles (Othmeni et al. 2024).

Foaming behaviour relies on a distinct balance between protein classes. The small, highly soluble albumin molecules diffuse rapidly to the newly generated air-water interface to provide immediate foamability and rapid volume expansion. In contrast, the larger globulin molecules migrate slower but structurally rearrange across a larger surface area, providing foam stability through steric hindrance and electrostatic repulsion between adjacent bubbles (Janssen et al. 2025).

Thus, albumins provide initial foaming capacity, while globulins provide stability, both required for proper foam in food application. (Janssen et al. 2025) further points out that there is an overall scarcity of research on the mechanisms that stabilises oat globulin and albumin foam.

2.4.3 Emulsifying Properties

Proteins are essential functional ingredients for stabilising food emulsions. Proteins act as physical emulsifiers by creating an amphiphilic barrier between the polar aqueous phase and the non-polar lipid phase (Burger & Zhang 2019). To inhibit emulsion failure, proteins must be structurally agile, allowing them to adsorb and unfold fast at the oil-water interface. By molecularly reordering, it provides a viscoelastic film that encapsulates oil droplets and prevents coalescence or flocculation (Lam & Nickerson 2013).

At pH values 4-7, native isolated oat proteins have low emulsifying capacity due to lower solubility; therefore, the emulsifying properties are enhanced at lower and higher pH. Emulsifying activity is improved for both OPI and OPC by succinylation and acetylation, due to increased access of functional groups originally folded within the protein molecular core. Consequently, modification of the native protein through altering the extrinsic factors results in better emulsifying properties (Kumar et al. 2021).

2.4.4 Rheological Profile

The rheological property of a system is defined by the shear viscosity for a fluid system. It tells how a fluid flows during processing and feels when consumed, experienced viscosity. Furthermore, a solid or semi-solid system is measured by elastic modulus (G') and fracture properties, which indicate the force required to break the gel structure. Together, these measurements are important in food application for product quality, shelf life and consumer experience (Badjona et al. 2025).

The ability of globular proteins to transition from a fluid state into a viscoelastic gel network is controlled by a combination of molecular forces, including hydrophobic interactions, hydrogen bonding, and covalent disulphide cross-linking (Badjona et al. 2025). Fluid viscosity serves as a direct indicator of molecular entanglement and internal friction.

The structural morphology of a globular plant protein gel is highly sensitive to the local electrostatic environment during network assembly. When a protein solution is heated or acidified close to its pI, the absence of net electrical charge eliminates repulsive forces, driving subunit clustering into a particulate network of spherical aggregates. This particulate structure gives macroscopically grainy mouthfeel and exhibits low moisture retention (Yang et al. 2017).

Conversely, assembling a gel network at a functional distance from the pI preserves a degree of electrostatic repulsion, forcing the denatured protein monomers to unfold into fine linear filaments. The filamentous structure is crucial for a smooth product and high-water holding capacity, since it entraps water better in the network than the particulate version (Yang et al. 2017).

2.4.5 Gelling Kinetics

During fermentation and processing, the interaction between oat and pea proteins produces distinct textural outcomes. The initial heating step unfolds the protein structures, enabling core hydrophobic regions to interact and assemble into aggregates first and a gel network if protein concentration is high enough (Tanger et al. 2022). Oat proteins possess a high concentration of sulphur-containing amino acids that resist thermal denaturation, which can limit their thermal self-gelation capacity in pure systems (Wang et al. 2023).

On the other hand, Brückner-Gühmann et al. (2021) highlight that it is possible to produce a heat-induced gel at both 90°C and 120°C, that is with and without thermal denaturation. This is further supported by the fact that disulphide bridges are not necessary for gel formation, although they contribute to gel stiffness in pea protein

gels (Tanger et al. 2022). While oat 12S globulins share structural similarities with high-performing gelling agents like soy glycinin, they exhibit very different aggregation kinetics. At high processing temperatures (110°C), some fraction of oligomeric oat globulin forms soluble aggregates, but further heating can cause isolated clusters instead of a continuous network (Mel & Malalgoda 2022b).

The pea protein denaturation enables aggregate formation, both soluble and insoluble, through non-covalent bonds or disulphide bridging, where 7S and 11S fractions aggregate interchangeably. The aggregation depends on sensitivity of the protein fractions for ionic strength, pH and temperature (Klost et al. 2020a).

Cold set gelation can be made by either salt or acid (Walsh et al. 2010). Gelation is dependent on protein solubility; lower solubility at acidic pH leads to a coarser network and higher water loss for pea protein gels (Klost et al. 2020b), similarly to oat. The final gel strength depends on a precise balance of attractive and repulsive forces. Syneresis will be seen if the attraction is too high; conversely, if attraction is too low, a gel will not be formed or phase separation might occur (Walsh et al. 2010).

Furthermore, addition of calcium (Ca^{2+}) is a common strategy to induce cold-set gelation in globular proteins, because it acts as a physical crosslink between the negative side chains on the surface of the proteins. By neutralising the electrostatic repulson it enables a network formation at lower temperature or at a pH value where the proteins otherwise would be soluble. Higher total concentration of protein and possible addition of Ca^{2+} to the protein solution will increase the G' (Maltais et al. 2005).

During the fermentation of oat protein-polysaccharide mixtures, the structural behaviour is governed by the pH-pI relationship. When operating at a pH below the protein pI induces and electrostatic assembly between positively charged polysaccharides, whereas operating above pI generates electrostatic repulsion (Walsh et al. 2010).

2.5 Production of Plant-Based Yoghurt

The manufacturing of fermented plant-based yoghurt analogues involves several processing interventions, which can be categorised into biological treatments (fermentation and enzymatic hydrolysis), chemical modifications (pH adjustment and mineral fortification), and physical texturing methods (homogenisation and ultrasonication).

The heating step in the production of milk analogues (hereafter “milk”) leads to starch gelatinisation and protein denaturation. For an oat ingredient like OPC, heating past the starch gelatinisation threshold provides essential baseline viscosity and physical suspension stability to the unfermented milk (Klost et al. 2020b).

A major biological challenge in plant matrices is that plant milks lack the chemical buffering capacity found in dairy systems. Consequently, during incubation of plant-based yoghurt, lactic acid bacteria experience an accelerated pH drop. This rapid acidification can inhibit regular bacterial multiplication rates and result in a lower total microbial count compared to traditional bovine milk yoghurts (Wang et al. 2023).

Furthermore, the rapid velocity of decreased pH directly dictates the protein aggregation patterns. While bovine dairy milk transitions into a highly ordered, characteristic honeycomb structure, the sudden shift in plant milks frequently generates alternative configurations. Fermented oat milk typically yields loose, disorganised particulate clumps, whereas soy matrixes organise into a denser “string of beads” micropattern (Wang et al. 2023).

2.5.1 Protein Blending for Structural and Nutritional Synergy

Including protein from different plant sources can enhance functionality in gelation, water-holding capacity and provide stable emulsions (Badjona et al. 2025). Blending cereal and pulse proteins enhances the nutritional profile by contributing with a complete amino acid profile. Cereals like oat are limited in the essential amino acid lysine, but rich in sulphur-containing amino acids (methionine and cysteine), whereas pulse proteins like pea are lysine-rich but limiting in sulphur amino acids.

Combining oat and pea proteins satisfies human nutritional requirements, generating a high-quality protein score according to Digestible Amino Acid Scores (DIAAS) and delivering a Protein Digestibility-Corrected Amino Acid Score (PDCAAS) similar to bovine dairy milk (PDCAAS = 1) (Spaen & Silva 2021; Dimina et al. 2022).

From a structural point of view, up to 80% of the pea protein fraction consists of globulin (Burger & Zhang 2019). A high globulin fraction can enhance the emulsifying performance by providing a robust viscoelastic film, hindering droplet coalescence (Makri et al. 2005). Furthermore, pea protein offers functional gelling characteristics similar to soy protein, while not being an allergen in comparison to soy (Burger & Zhang 2019).

2.5.2 Functional Role of Dietary Fibre

The inclusion of native or added dietary fibres provides essential structural reinforcement to plant-based yoghurt gels. The hydrocolloidal property of fibres increases water binding capacity which restricts serum mobility within the continuous matrix. Furthermore, fibres impose steric hindrance at the interface, which help stabilise emulsions and increase the storage modulus (Xu 2012). Additionally, it provides higher viscosity and a matrix filling effect, reducing post-fermentation syneresis.

2.5.3 Ultrasonic Treatment

Ultrasonication represents a non-thermal green technology capable of modulating protein conformation without altering primary amino acid sequences. The application of high-intensity ultrasound induces acoustic cavitation, which generates intense local shear forces that disrupt hydrogen bonds and hydrophobic clustering. This technique is used to fragment large, insoluble protein aggregates into smaller, dispersed units (Shanthakumar et al. 2022).

The secondary, tertiary and quaternary structures of the protein are altered, subsequently surface area increase together with exposed hydrophobic domains. Consequently, ultrasonication accelerate interfacial adsorption, thus improving foaming and emulsifying properties (Xu et al. 2023).

3. Materials and Methods

The experimental workflow was conducted as follows: protein dispersions containing OPC and PPI were prepared and subjected to ultrasonication to investigate the functional and structural properties of the raw materials prior to analysis. Subsequently, milk analogues were prepared using OPC, PPI and/or $CaCl_2$, followed by yoghurt fermentation and rheological measurements.

All analyses were performed in triplicate (technical replicates) at room temperature unless otherwise stated. No statistical analysis was performed. Therefore, the results should be interpreted as indicative trends rather than statistically significant differences.

3.1 Raw Materials and Sample Preparation

The two protein sources used in this study were OPC PrOatein (batch number 2511040044) supplied by Lantmännen and PPI Vestkorn Pisane XS01. An overview of the composition of the two raw materials, according to the supplier specifications, is given in Table 1. The protein ratios used were (OPC) 1:1, (OPC:PPI) 90:10, and (OPC:PPI) 75:25.

Table 1 Composition of the raw materials OPC and PPI according to the specifications provided by the supplier.

Nutrient	OPC PrOatein (%)	PPI Pisane (%)
Fat	13	1.3
Carbohydrate	22	0.4
Fibre	4.6	2.3
Protein	55	83.6
Salt	0.1	2

Further, commercially available rapeseed oil, calcium chloride ($CaCl_2$; CAS 10043-52-4, Labkem, Cach-02A-500), and water were used in the milk analogue and subsequent yoghurt formulations.

Oat milk samples were prepared by blending 91 g of the protein ratios with 10 g of sucrose and 909 g of water. For the calcium milk samples, a $CaCl_2$ stock solution was prepared by dissolving 7.35 g $CaCl_2$ in a 50 mL volumetric flask. The two $CaCl_2$ concentrations evaluated were 30 mM and 100 mM in the milk and yoghurt analogues.

The mixtures were stirred using a magnetic stirrer for 30 min to ensure complete dispersion of ingredients. 19 g of rapeseed oil was subsequently added before homogenisation using an Ultra Turrax T25 (Janke & Kunkel IKA- Labortechnik, Staufen, Germany) at 13500 rpm for 5 min. The samples were then sonicated according to the procedure described in Section 3.2.

The oat milk samples were heat-treated at 90°C for 5 min and cooled to room temperature. A portion of each sample was used for rheological measurements. The remaining portion was divided into 30 mL samples, inoculated with 120 µL yoghurt starter culture (Vega^{TM} Mild CHR Hansen) and incubated at 40°C until reaching pH 4.4. The pH was measured hourly using a calibrated pH meter.

3.2 Methods

To determine the functional properties of the raw materials, including water-holding capacity, water solubility index, foaming properties, and emulsification properties, the raw materials as well as different protein ratios were prepared accordingly. Furthermore, to verify the effect of ultrasound pretreatment on the functionality of the proteins, the samples were subjected to ultrasonication (Sonics VCX-750 Vibra-Cell, Sonics & Materials Inc., Newtown, USA) following an adjusted method described by (Hu et al. 2015 and Xiong et al. 2018). Ultrasound treatment was performed at 100% amplitude (500 W) in 1 min cycles until aggregate disruption was observed (approximately 3 min). For each treatment, 50 mL of protein suspension was placed in a beaker immersed in an ice bath to prevent overheating.

3.2.1 Light Microscopy (LM)

To visualise the effect of ultrasound treatment on protein microstructure, light microscopy (Nikon, Eclipse Ni–U microscope, Tokyo, Japan) equipped with a 10 × (0.75 NA) apochromatic objective was used. 250 µL of dispersion was added together with 100 µL of light green (Sigma Aldrich, L1886) in an Eppendorf tube and homogenised by gentle pipetting. A droplet of the stained sample was analysed, and micrographs were acquired with a Nikon Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan) with 0.12 µm/pixel.

3.2.2 Water Holding Capacity (WHC) and Water Solubility Index (WSI)

Protein suspensions were prepared at a protein-to-water ratio of 1:10 and mix for 30 minutes using a magnetic stirrer. WHC was determined using a method adapted from Varayil & Mitra (2026). Protein dispersions were added to pre-weighed Falcon tubes, which were subsequently weighed. Samples were centrifuged for 10 min at 12.000 g, followed by decanting of the supernatant (used for WSI analysis). The remaining pellet was weighed. WHC was expressed as g water retained per g protein and calculated using the following equation:

Wi=Initial protein weight = dry mass of protein used in suspension
Wf=Final pellet weight = mass of hydrated pellet after centrifugation

$$WHC, g/g = \frac{Wi - Wf}{Wi}$$

WSI was measured based on a method outlined by Varayil & Mitra (2026). The supernatant from WHC, Section 3.2.1, was dried (95°C, overnight) and dry solids were weighed. WSI was calculated accordingly:

$$WSI, \% = \frac{weight\ of\ dry\ solids\ from\ supernatant}{initial\ weight\ of\ sample}$$

3.2.3 Foaming Properties

Foaming properties of OPC with PPI mixtures at different ratios, with and without sonication, were determined using a modified method based on Yang et al. (2023) and Varayil & Mitra (2026). Protein dispersions (10 mg/mL) were stirred magnetically for 30 min to ensure solubilisation.

The initial solution volume (V1) was measured in a graduated 100 mL cylinder prior to homogenisation. Samples were then homogenised using an Ultra Turrax T25 (Janke & Kunkel IKA- Labortechnik, Staufen, Germany) at 13.500 rpm for 2 min.

The foam volume immediately after homogenisation (V0) and after 30 min (V2) was recorded. Before each measurement, the cylinder was gently rotated once to allow the foam to settle uniformly.

Foaming capacity (FC) and foam stability (FS) were calculated as follows:

$$FC, \% = \frac{V0}{V1} \times 100$$

$$FS, \% = \frac{V2}{V0} \times 100$$

3.2.4 Emulsifying Capacity

Emulsifying capacity (EC) was determined using a modified method described by (Yang et al. 2023). Protein solutions were prepared (10 mg/mL) and stirred magnetically for 30 min.

Emulsions were prepared by adding 9 g rapeseed oil per 21 mL protein dispersion using an Ultra Turrax T25 (Janke & Kunkel IKA- Labortechnik, Staufen, Germany) at 24.000 rpm for 5 min. In samples where foam formation occurred, the beakers were gently tapped to release entrapped air before measurements were performed. After 5 min, the height of the cream layer (H_c) and the total height of the emulsion (H_t) were measured.

EC was calculated by using the following equation:

$$EC, \% = \frac{\mathrm{Hc}}{Ht} \times 100$$

3.2.5 Rheological Measurements

To determine the rheological properties of the final milk analogues before and after fermentation, the milk and yoghurt samples were prepared according to Section 3.1. Rheological measurements were performed using a Discovery HR-3 rheometer (TA Instruments, New Castle, DE, USA) equipped with a 40 mm aluminium plate (geometry number 112471) and a gap size of 1 mm. The viscosity was recorded during a flow sweep across a shear rate interval of 0.01-150.0 s^{-1} at a constant temperature of 25 °C. Soak time was set to 30 s.

3.2.6 Confocal Laser Scanning Microscopy (CLSM)

To determine the microstructure of the fermented yoghurt samples, confocal laser scanning microscopy (CLSM) was used. Samples were stained using fluorescein isothiocyanate (FITC) for starch, Rhodamine B for protein, and Calcofluor White for dietary fibre, resulting in green, red, and purple fluorescence signals, respectively. The corresponding excitation wavelengths were 488 nm for FITC, 568

nm for Rhodamine B, and 410 nm for Calcofluor White. All images were acquired at a resolution of 1024 × 1024 pixels and subsequently processed using ImageJ.

4. Results and Discussion

4.1 Ultrasonication Effect on Particle Size

Structural analysis via light microscopy demonstrates the effect of ultrasonication on particle size reduction across all protein blends (Figure 1). The micrographs illustrate a distinct morphological transition from large, dense aggregates visible in the control samples (C + sample name) into a finely dispersed protein matrix in the ultrasound-treated samples (U + sample name). Specifically, the ultrasonicated Pea formulations exhibit a finer protein network than ultrasonicated Oat. The inclusion of PPI appears to result in a more cohesive and less coarse matrix compared to ultrasonicated Oat.

The particle size reduction is attributed to acoustic cavitation, which generates intense local forces that disrupt protein clusters (Shanthakumar et al. 2022). The finer particle matrix in samples with PPI implies that PPI is more susceptible to sonication-induced fragmentation than the OPC matrix.

One apparent difference between oat and pea samples is the level of protein purity in an isolate compared to a protein concentrate. In the concentrate, there are likely starch-protein complexes (Laursen et al. 2024), which provides structural rigidity, thereby shielding the proteins from full the impact of ultrasonic waves. In contrast, the aggregates in the PPI are primarily stabilised through non-covalent bonds, which are more easily disrupted.

Furthermore, the disruption of these macromolecular complexes in ultrasonicated Oat likely explains the observed increase in solubility by increased surface area (Laursen et al. 2024), which is further supported by the higher water solubility index (WSI) values for all ultrasonicated samples, see Section 4.2.

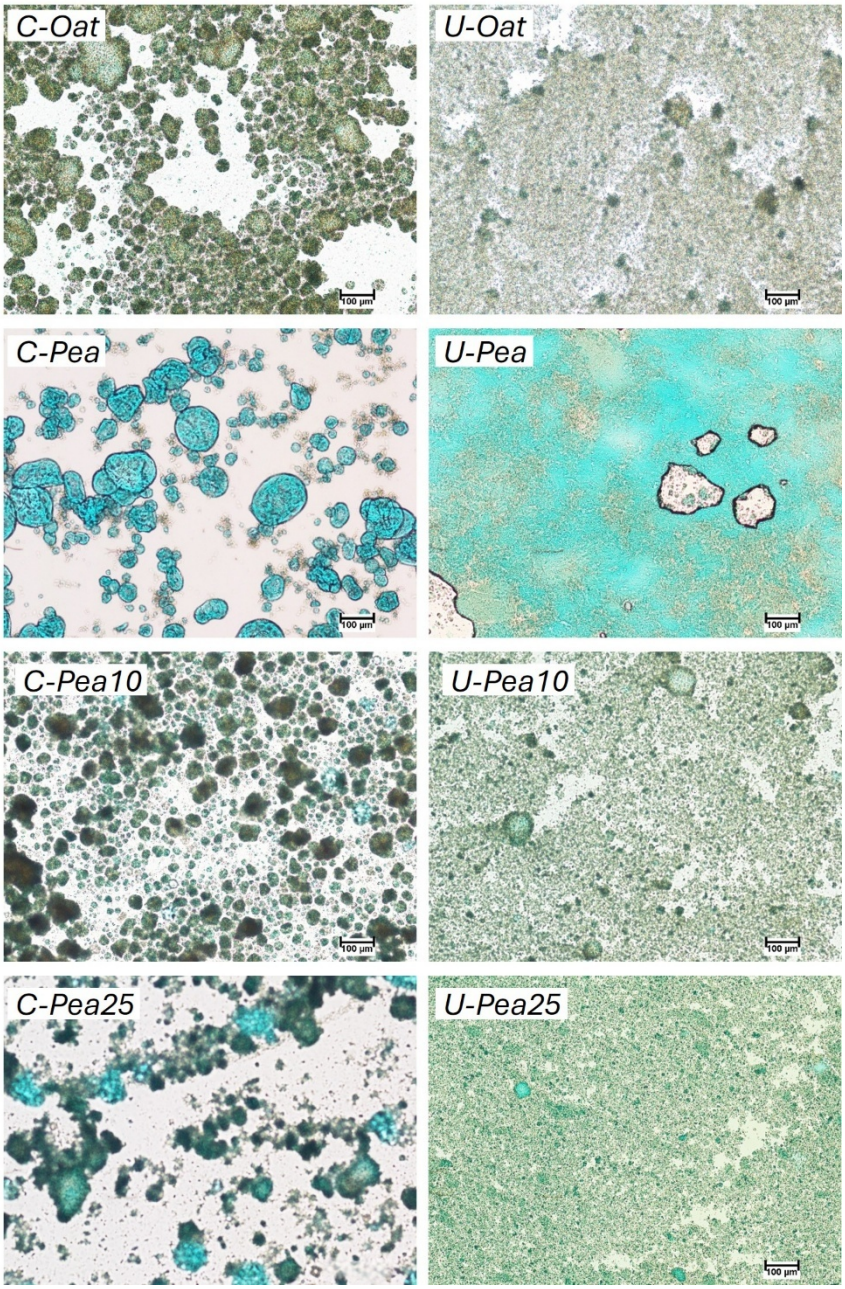

Figure 1 Light microscopy micrographs of plant protein blends illustrating the effect of acoustic sonication on matrix particle size distribution (100 µm scale bar used as reference).

4.2 Water Solubility Index and Water Holding Capacity

The WHC for the control samples ranged between 1.46-1.73 g/g, with highest capacity in Pea25 and lowest in Pea10 (Figure 2). For the ultrasonicated samples, the calculated WHC values were lower, ranging from 0.88 to 1.27 g/g, with Pea25 holding the most water and Oat the least.

Regarding the water solubility index (WSI), the ultrasonicated samples exhibited higher WSI values (28.3–29.1%) than the control samples (26.3–26.6%). Among the ultrasonicated samples, Pea10 showed the highest WSI, whereas Oat showed the lowest. Within the control samples, both Pea10 and Pea25 exhibited a WSI of 26.6%; however, the variability differed between the samples. Control Pea10 showed a lower standard deviation (0.1) than control Pea25 (0.5), while control Oat exhibited the greatest variability overall (1.0).

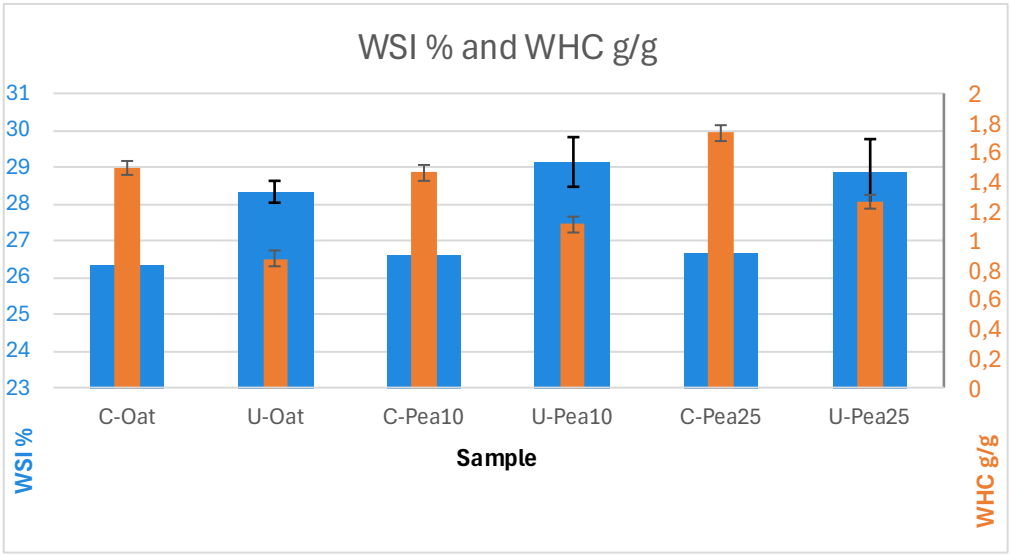

Figure 2 WSI % and WHC g/g of the protein blends with and without sonication. Blue bars represent WSI and orange bars represent WHC. Values are presented as mean ± SD (n=3), with error bars indicating the standard deviation of three technical replicates.

During the experimental process, a distinct difference in the supernatant clarity was observed. As shown in Figure 3, control samples exhibited clear phase separation, while ultrasonicated sample supernatants remained turbid even after extending centrifugation times to 40 minutes for Pea and 70 minutes for Pea25.

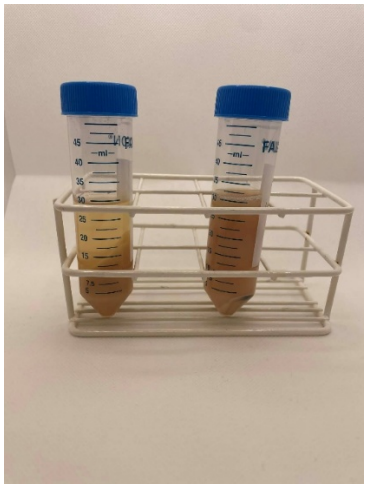

Figure 3 Comparison of supernatant turbidity after centrifugation. Left tube is C-Pea with clear supernatant and right tube is U-Pea with turbid supernatant.

The results on WHC for the ultrasonicated samples must be interpreted alongside the WSI data. The persistent turbidity in ultrasonicated samples suggests that increased solubility due to sonication effect on reduced particle size (Figure 1) prevented the ultrasonicated samples from pelleting during centrifugation. Consequently, some of the dispersed powder was decanted together with the supernatant.

Since the powder did not fully precipitate, the calculated WHC values for ultrasonicated samples are likely underestimated and do not accurately represent the actual hydration properties of the material. Thus, the high solubility achieved by sonication limits the reliability of the results from centrifugation method for characterising ultrasonicated samples.

The increased solubility from ultrasonication aligns with Shanthakumar et al. (2022) who noted that ultrasonication improved PPI solubility by protein structure disruption. While the control WSI (approximately 26.6%) are comparable to the 33% reported for native dry-milled oat protein (Jiang et al. 2015), they are substantially higher than values reported for oat flour, which range from 3.73 to 11.08% (Choi et al. 2012; Šimurina et al. 2018).

The WHC of the control samples (1.46-1.73 g/g) falls within the expected range of oat-derived material (1.33-3.35 g/g) (Ponnampalam et al. 1988; Guan et al. 2007; Choi et al. 2012; Šimurina et al. 2018). Furthermore, the highest WHC with 25% PPI added is consistent with studies on pulse protein concentrates, where values typically vary between 1.8 to 2.5 g/g (Varayil & Mitra 2026), however Oat (1.50 g/g) and Pea10 (1.46 g/g) share approximately the same baseline value. This indicates that while protein type influences the basic capacity of WHC and WSI,

the processing method (sonication) seems to be the primary driver dictating the final hydration properties.

4.3 Foaming Properties

The foaming capacity (FC) was considerably higher in all ultrasonicated samples compared to the control samples (Figure 4). Among the ultrasonicated samples, the highest FC was observed in Pea25 (45.0 %), followed by Pea10 (23.3 %), while Oat displayed the lowest value (18.3 %). In comparison, the control sample values were notably lower (6.7 to 11.7 %), although the general trend across sample types remained consistent.

Foaming stability (FS) was negligible for most of the samples after 30 minutes, except for ultrasonicated Pea25, with a FS value of 11.2 %. The improved capacity and stability observed at higher PPI percentage suggests that combining a dense protein concentration with ultrasonication treatment likely promotes a more resilient viscoelastic film at the air-water interface.

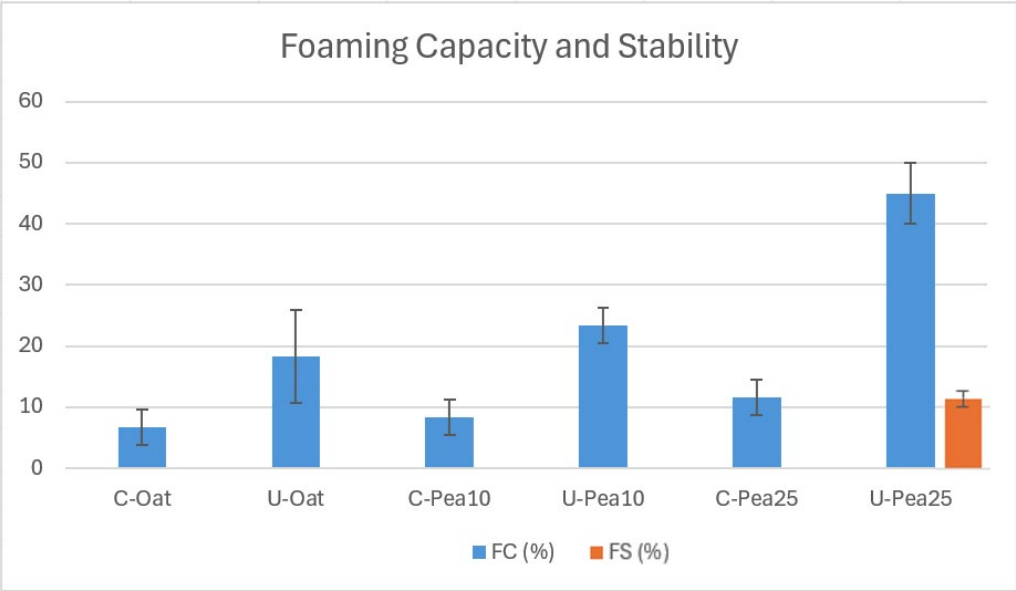

Figure 4 Foaming capacity and stability of protein blends with and without sonication. Blue bars represent FC and orange bar represent FS. Values are presented as mean ± SD (n=3), with error bars indicating the standard deviation of three technical replicates.

Notably, ultrasonicated Pea25 exhibited nearly double the FC compared to Pea10. This trend indicates that including PPI considerably enhances the FC of the base OPC system, especially when combined with ultrasonication processing. Which is in line with a study by (Shanthakumar et al. 2022) where sonication improved PPI

foaming properties. Considering that ultrasonicated Oat and Pea10 displayed quite similar FC values compared to Pea25, it appears that a higher PPI percentage (above 10%) is required to achieve improved air-water interface stabilisation. Additionally, the larger particle aggregates observed in all control samples (Figure 1) indicate a slower and less efficient migration and adsorption to the interface (Janssen et al. 2025).

Comparing these results to other protein sources (Table 2), it is apparent that the values correspond closely with those of wheat flour, wheat/chickpea flour blends, and native oat protein. Depending on the application, these oat-pea blends would not be suitable direct substitutes for high-performance proteins like egg white (FC 200-317%) in applications such as meringues.

However, the potential application of these blends is highly dependent on the specific functional requirements of the food product. While most samples lack sufficient FS for a 30 min duration, they might still perform well in applications where long-term stability is not critical. For instance, a coffee foam layer does generally not require the 30-minute stability tested in this study. Thus, these sonicated blends remain viable candidates for food applications where moderate foaming and rapid consumption are expected.

Table 2 Foaming capacity and stability of different protein sources for comparison. Values represent mean values and are not directly comparable due to differences in sample preparation and experimental conditions between studies.

Protein source	FC (%)	FS (%)	source
Natural egg white	317	91	Zhang et al. 2024
Egg white	200	33	Lomakina & Míková 2006
Milk protein (sodium caseinate)	91.5	41.5	Stamatie et al. 2021
Oat bran protein concentrate	126.5	63.0	Guan et al. 2007
PPI	79.5	29.5	Stamatie et al. 2021
Oat protein	48.5	40.5	Stamatie et al. 2021
Wheat + Chickpea flour	23.43	85.90	Nawaz, H. et al. 2015
Wheat flour	11.76	93.85	Nawaz, H. et al. 2015

4.4 Emulsifying Capacity

The emulsifying capacity (EC) was notably higher in the ultrasonicated samples compared to the control samples across all protein blends (Figure 5). Within the control group, Oat (38 %) demonstrated the lowest EC, followed by Pea10 (44 %) and Pea25 (63 %). A similar trend was observed in the ultrasonicated samples, where Oat (49 %) presented the lowest EC, while Pea10 and Pea25 reached 56 % and 75 %, respectively.

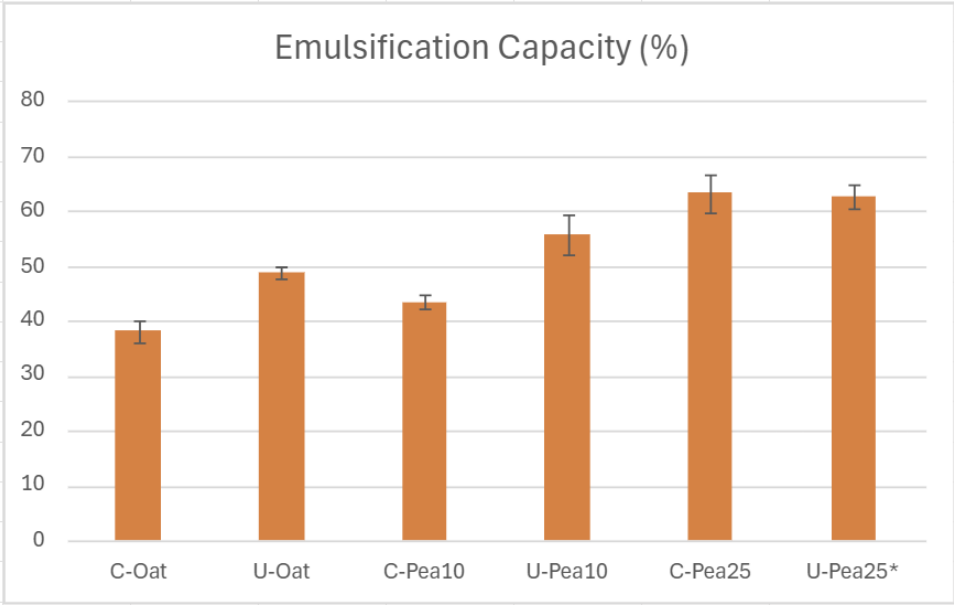

*Figure 5 Emulsification capacity (%) of plant protein blends with and without sonication treatment. Values are presented as mean ± SD (n=3), with error bars indicating the standard deviation of three technical replicates. *U-Pea25 parameters represent a duplicate instead of a triplicate.*

The general trend of increased EC in ultrasonicated samples aligns with Shanthakumar et al. (2022) who reported that sonicating PPI increases emulsifying activity. This improvement is likely attributed to particle size reduction and exposure of hydrophobic amino acid residues induced by ultrasonication. These structural changes facilitate rapid protein adsorption at the oil-water interface, creating a viscoelastic film that prevents oil droplet coalescence (Lam & Nickerson 2013). Furthermore, as pointed out by Mel & Malalgoda (2022), the increased solubility observed in ultrasonicated samples (see WSI result section 4.2) is a key determinant for effective film formation, as soluble proteins migrate more efficiently to the interface.

The EC values achieved here (38 to 75%) are partly competitive with some existing literature, for instance Yang et al. (2023) reported EC values ranging from 28-52% for different pea protein genotypes at the same concentration (10 mg/ml). However, it is important to note that protein functionality is highly dependent on extrinsic factors such as pH, ionic strength and the presence of other solutes like salts or sucrose. Consequently, this measurement should be viewed as relative to the specific experimental setup in this study.

A study referenced by (Mel & Malalgoda 2022b) reported that oat fractions had better emulsify and fat binding properties in comparison to soy. This is contrary to the results of this experiment, where the inclusion of PPI increased EC of the OPC base. However, the apparent difference in this test might rather be a result of the aggregated, complex clusters in OPC base material than the actual structural performance of the oat protein itself. For instance, the inclusion of PPI seems to affect EC more than the sonication treatment, as ultrasonicated Oat (49%) has notably lower EC than control Pea25 (63%).

4.5 Rheological Properties

Note on data presentation: Standard deviations of the triplicate measurements for rheology were removed from the curves to improve graphical readability and are provided in full within Appendix.

4.5.1 The Effect of Pea Protein Addition on Milk and Yoghurt System

The viscosity behaviour of the pure oat and oat-pea milk systems before and after fermentation is illustrated in Figure 6. Based on this the pea-containing milk systems (Pea10 and Pea25) exhibited a near-zero yield stress and appeared less resistance to stress than the pure oat blend (Figure 7). Increasing the PPI concentration from 10% to 25% resulted in only a minor increase in fluid viscosity, suggesting a limited contribution of pea proteins to the bulk structure under the tested conditions.

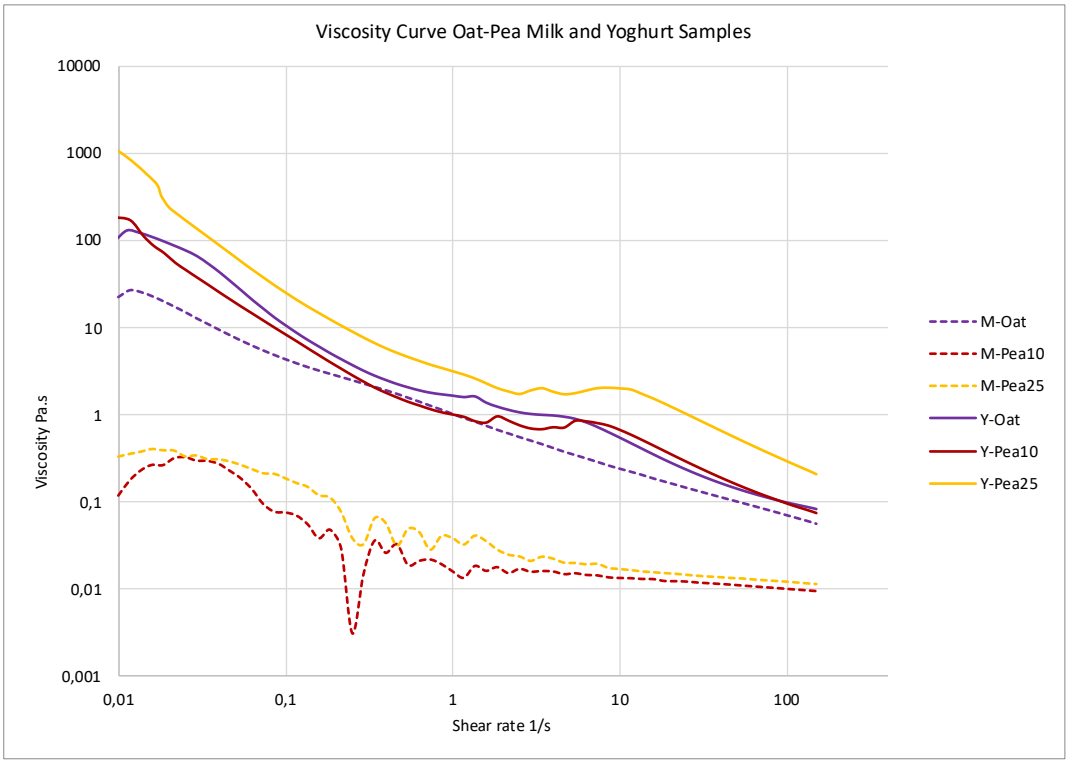

Figure 6 Viscosity curves of unfermented oat-pea milks and their corresponding fermented yoghurts as a function of shear rate.

Following fermentation, the structural properties inverted. Among all fermented formulations, the Pea25 yoghurt showed the highest shear stress across the tested shear rate range, developing the strongest structural network and highest flow resistance (Figure 7). Notably, Pea25 was the only formulation that transitioned from liquid to gel consistency after fermentation, demonstrating a higher yield stress of approximately 24 Pa. Its apparent viscosity at low shear rates was also markedly higher than all other samples (Figure 6).

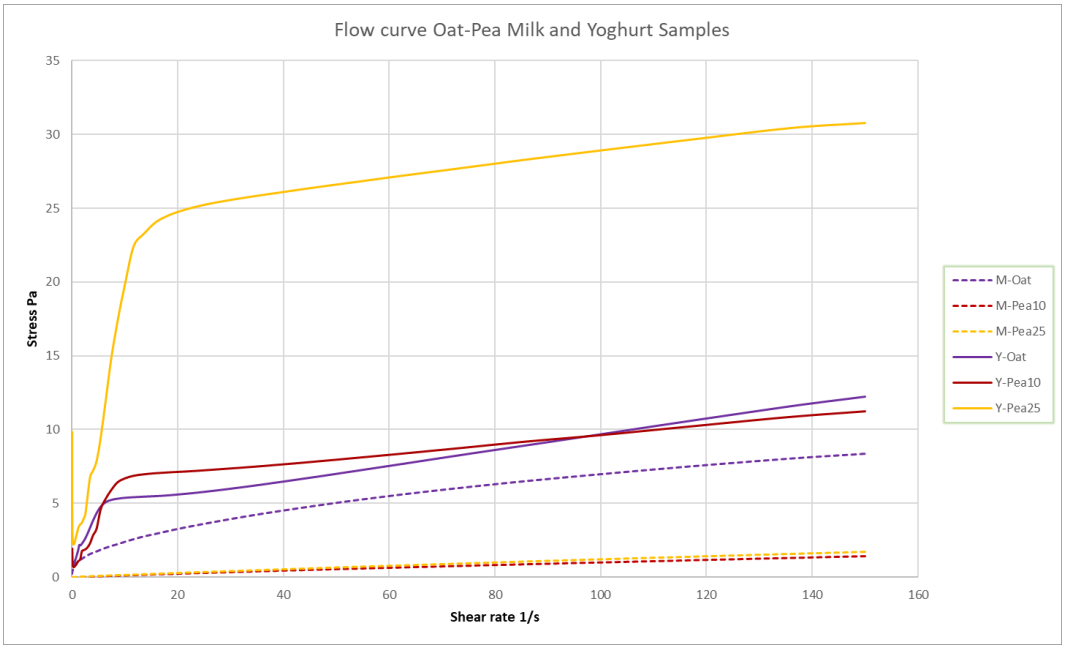

Figure 7 Flow curves of oat-pea milks and yoghurts demonstrating shear stress resistance as a function of shear rate.

The distinct behaviours of the pea protein blends at different processing stages could be explained by the shifted electrostatic charge of the molecules. During fermentation, microbial metabolic activity made pH drop from the initial pH of the milk for each sample to an acidic pH endpoint. Notably, the final pH of the fermented pea-blends was slightly higher than that of the pure oat systems, see Figure 8.

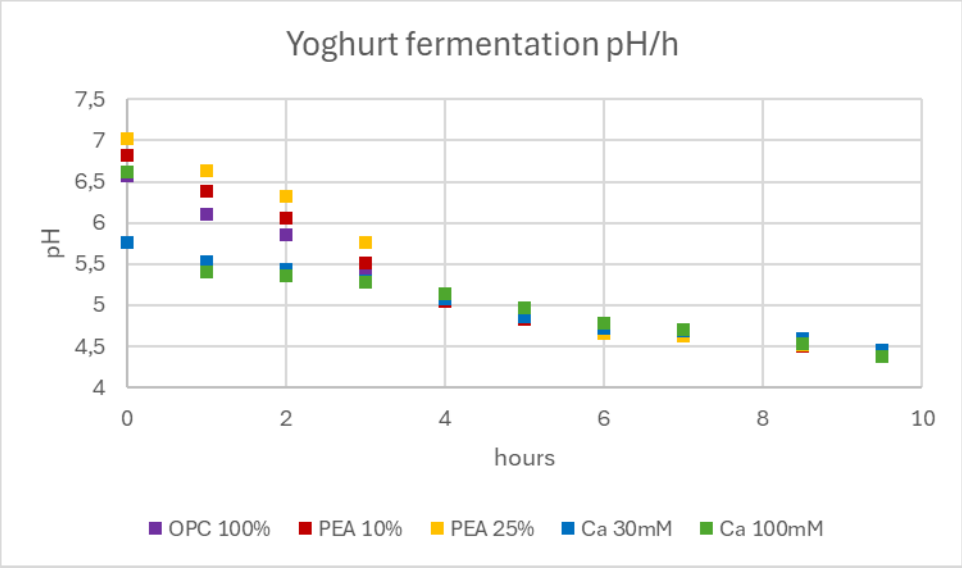

Figure 8 pH profile changes as a function of fermentation time at 40°C.

Furthermore, the macronutrient profile of the milk systems reflected the amount of added PPI (Table 3), with the Pea25 sample exhibiting the highest protein content and lowest carbohydrate and fat levels. This compositional shift partly explains the lower viscosity of the pea-containing blends. The dilution of OPC reduced the concentration of gelatinised starch, thereby weakening the structural matrix.

At the observed neutral pH values of these unfermented milks, the higher percentage of pea proteins likely amplified electrostatic repulsion. Because this pH is far from their isoelectric point (pI 4.5-4.8 depending on genotype), the pea proteins carry a strong negative charge (Daba & Morris 2022). This state prevents the proteins from forming a network. Instead, the individual protein chains likely interfere with the structural alignment of the oat starch, fibre, and protein, reducing internal friction and lowering bulk viscosity in the pea milk systems.

Table 3 Nutrient composition of the different protein blends in oat milk formulations.

Sample	Fat (%)	Carbohydrates (%)	Fibre (%)	Protein (%)	Salt (%)
Oat	3.0	2.9	0.4	4.9	0.0
Pea10	2.9	2.7	0.4	5.1	0.0
Pea25	2.7	2.4	0.4	5.5	0.1

Conversely, as fermentation drives the pH down towards the pI, electrostatic repulsion diminishes, which allows the milk system to transform into an acid-induced pea protein gel (Brückner-Gühmann et al. 2019b). As illustrated by the flow and viscosity curves (Figure 6 and Figure 7), PPI exhibit no thickening capacity at neutral pH but acts as a gelling agent under acidic condition. Consequently, the higher protein concentration in the Pea25 formulation seems to be the primary factor for increased viscosity compared to the other samples.

Because the milks with Pea inclusion exhibited the lowest initial milk viscosities but the highest final yoghurt strengths, gelation cannot be attributed to OPC starch gelatinisation alone. Rather, it was primarily driven by the development of the acid-induced protein network.

While Brückner-Gühmann et al. (2019b) stated that OPC-based yoghurts are primarily stabilised by a starch gel, the findings of this study indicate that starch gelatinisation alone may not fully explain the observed rheological behaviour. This is evidenced by the fermented yoghurts exhibiting higher stress resistance than their corresponding unfermented milks (Figure 7). This further suggests that the concentrations or microstructural arrangement of starch, fibres, and proteins of the OPC formulation are insufficient to establish a continuous matrix on their own at the tested protein concentration.

Nevertheless, because Pea10 displayed rheological properties closer to the unfortified oat control than Pea25, a certain concentration of PPI is required to achieve a continuous gel matrix. A 10% added PPI is insufficient to form a proper yoghurt like texture, whereas the 25% inclusion meets or exceeds this critical gelation threshold for a yoghurt with structural integrity based on this OPC product.

The structural outcomes can be contextualised by examining how plant proteins behave under thermal and acidic conditions. Brückner-Gühmann et al. (2021b) observed that at pH 4.5, reduced electrostatic repulsion near the pI led to oat protein isolate (OPI) gels characterised by aggregates rather than a filamentous network, regardless of whether they heated to 90°C or 120°C. However, processing at 90°C yielded a texture like yoghurt, whereas 120°C generated higher overall gel strength.

Furthermore, according to Wang et al. (2023), soy-based yoghurts frequently exhibit higher gel strength than pure oat-based yoghurts due to stronger intermolecular crosslinking capacity. Considering the similarities between soy and pea proteins (Klost et al. 2020a), this crosslinking mechanism likely explains why Pea25 outperformed Oat. Pulse 7S and 11S globulin are related to gelling properties, however, oat globulins consist primarily of 12S globulin, exhibiting different aggregation kinetics.

The finding that PPI-fortified yoghurts exhibited higher yield stress than the pure oat yogurt is consistent with Wang et al. (2023a). They reported that soy-based yoghurt possessed higher firmness, WHC, and a higher yield point (31.31 Pa) than both reconstituted milk yoghurt (0.56 Pa) and pure oat yoghurt (0.22 Pa). Homogenous oat gelation may be structurally hindered by insoluble oat protein aggregates that cause steric hindrance (Ben-Harb et al. 2018). This effect is linked to the glutamine-rich surfaces of oat globulins, which make them more hydrophobic compared to other plant globulins (Spaen & Silva 2021), favouring self-aggregation over continuous matrix formation.

Analysis of Yoghurt Microstructure

Microstructural analysis via Confocal Laser Scanning Microscopy (CLSM) revealed the physical basis for the observed rheological differences in the fermented samples (Figure 9). The Pea25 sample developed a dense, continuous protein matrix where starch granules were thoroughly embedded within the structural network compared to the open, clustered arrangement of Oat. The tighter network resulted in improved water entrapment.

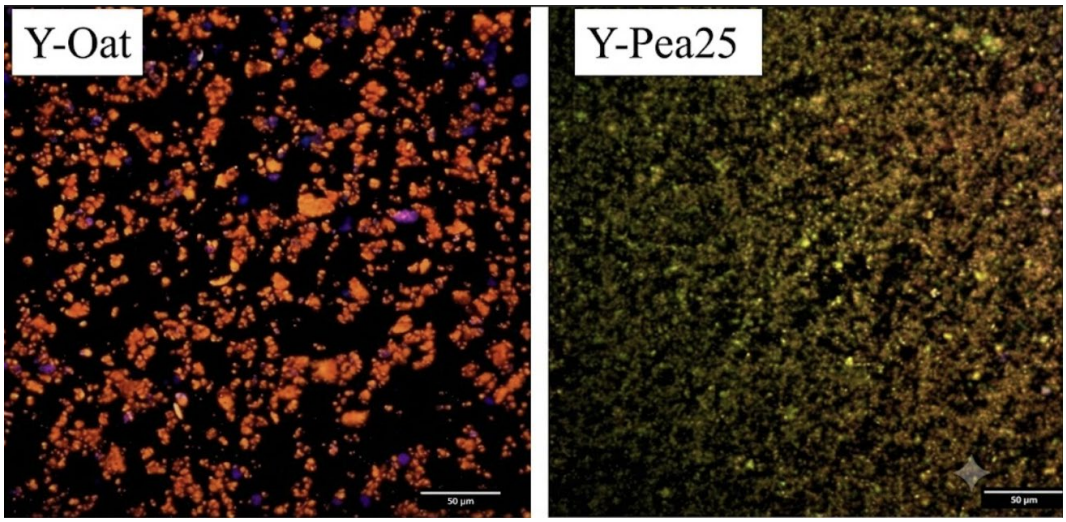

Figure 9 CLSM micrographs of Y-Oat and Y-Pea25 yoghurt samples visualising protein (red), starch (green), and dietary fibre (purple) distributions.

Walsh et al. (2010) previously demonstrated that OPI has a limited capacity for thermal self-gelation and typically requires the addition of a supplementary gelling agent (such as polymerised whey protein tested in their study) to establish a cohesive matrix. A parallel can be drawn to this study where the base milk underwent thermal processing, but it was the combination of acid fermentation and the 25% PPI fortification that supported and reinforced the gelling properties of the OPC system.

Ultimately, this high-density matrix, combined with increased particle friction and continuous phase entrapment, provides a clear microstructural explanation (Figure 9) for the elevated yield stress and apparent viscosity measured in Pea25. These results align with Wang et al. (2023), who observed that a dense, fine network correlates to higher storage moduli (G') and structural firmness. In their study, soy yoghurts exhibited substantially higher firmness (75.75 ± 1.79) than oat yoghurts (9.99 ± 0.17), which displayed a loose, uneven structure. This structural firmness directly dictates moisture retention, where a stiffer gel resists compression during centrifugation better than a loose, soft gel, reducing water release (Xia et al. 2022).

4.5.2 The Effect of Calcium Addition on Milk and Yoghurt System

The viscosity of the pure oat and $CaCl_2$ fortified milk systems prior to and after fermentation is presented in Figure 10. Among the unfermented milk samples, the control Oat and the Ca100 displayed the highest apparent viscosities across the measured shear range, indicating greater baseline structural integrity. Both samples

shared a pH of 6.6 (Figure 8), a state that appears to support stable intermolecular interactions and network suspension prior to fermentation.

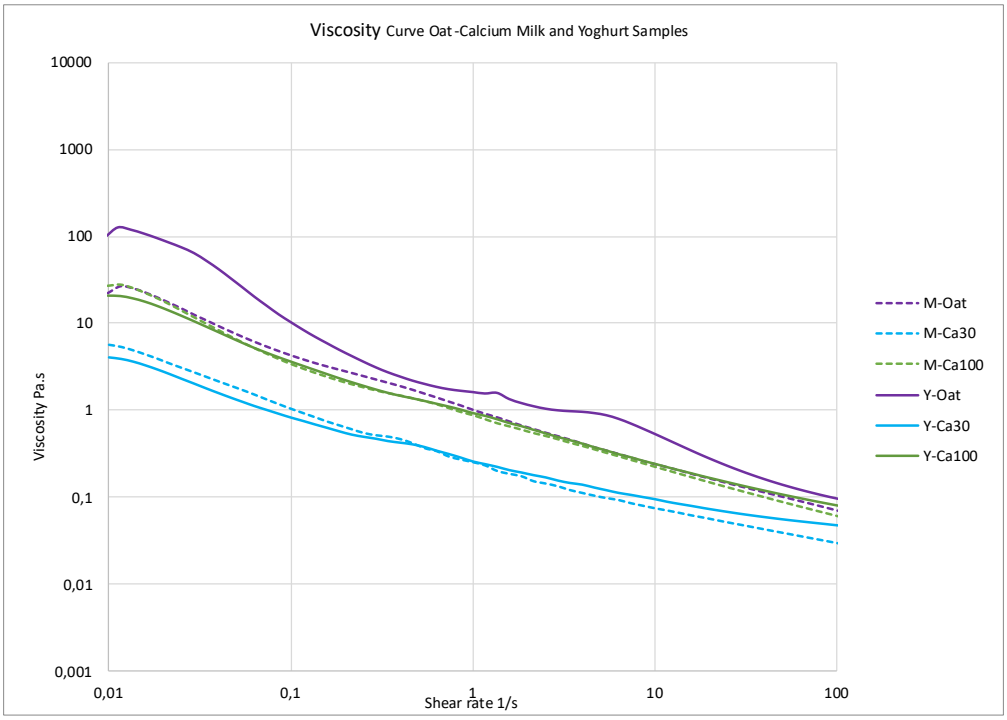

Figure 10 Viscosity curves of unfermented oat-calcium milks and their corresponding fermented yoghurts as a function of shear rate.

In contrast, the addition of 30 mM $CaCl_2$ resulted in a pronounced decrease in fluid viscosity for the Ca30 (Figure 10), which was accompanied by a lower pH of 5.8 (Figure 8). This implies that at this specific ionic strength and reduced pH, calcium addition induces a destabilisation effect among the suspended polymer components, weakening the overall structure and reducing fluid viscosity as shown in Figure 10 and overall flow resistance as shown in Figure 11.

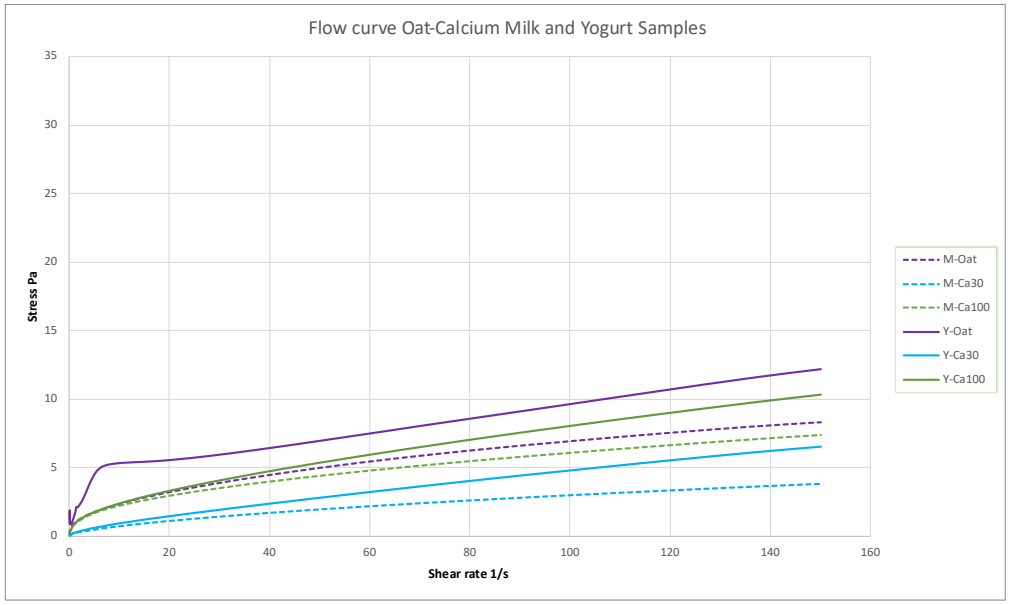

Figure 11 Flow curves of unfermented oat-calcium milks and their corresponding fermented yoghurts as a function of shear rate.

Following fermentation, the calcium-supplemented oat-based systems exhibited lower shear stress values compared to the unfortified control Oat (Figure 11). Among these, the Ca30 yoghurt sample displayed the lowest flow resistance overall, demonstrating that adding $CaCl_2$ under these processing conditions actively weakens rather than reinforces the structural network of the final fermented yoghurt based on this OPC product.

The fermented Ca samples exhibited lower structural integrity, with no clear yield stress in comparison to Oat (4.5 Pa), which also exhibited higher resistance to flow and viscosity. The Ca30 showed lower resistance to flow than Ca100 Figure 11. Furthermore, the $CaCl_2$ addition did not show a linear relationship with gel strength. This reduction in shear stress in the calcium-containing samples suggests divalent ionic interactions alter the spatial organisation of proteins and polysaccharides. Rather than reinforcing the matrix via salt bridges, the salt addition under these conditions might have contributed to a salting-out effect which led to partial destabilisation of the microstructure.

Analysis of Yoghurt Microstructure

CLSM visualised the microstructural matrix reflecting the rheological properties of each corresponding sample (Figure 12). The control Oat sample demonstrated distinct, isolated structural clusters within a weakly interacting network, showing visible aqueous phases trapped between micro-particles. In the Ca100 sample,

however, these clusters were larger, more pronounced particulate aggregates than in Oat. Consequently, Ca100 displayed a lower network continuity.

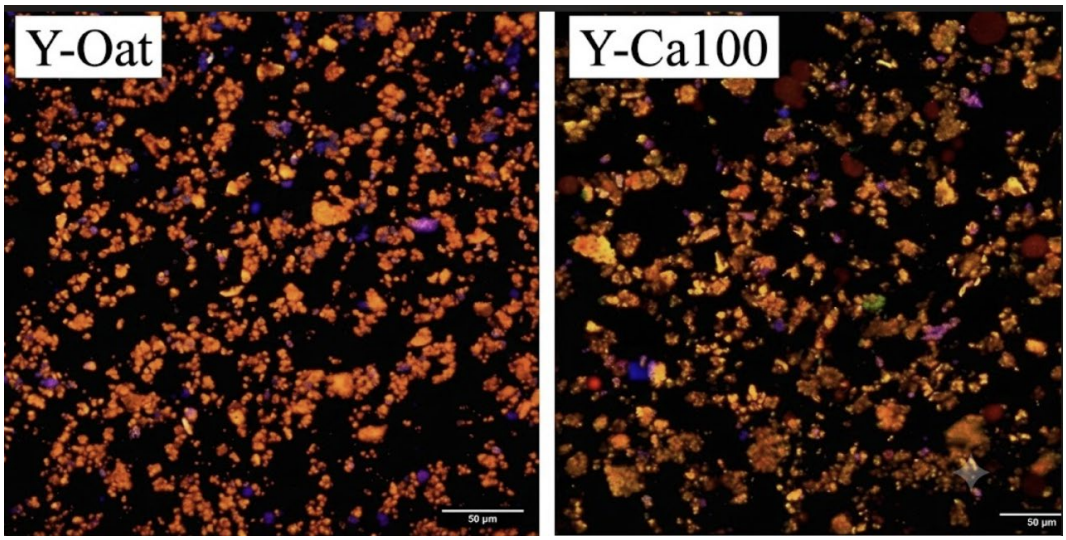

Figure 12 CLSM micrographs of Y-Oat and Y-Ca100 yoghurt samples visualising protein (red), starch (green), and dietary fibre (purple) distributions.

The microstructural arrangement of Oat (Figure 12) indicates that there was limited protein-protein interaction outside of these isolated clusters, preventing the formation of a cohesive, uniform gel and resulting in the low viscosities observed during testing.

The addition of 100 mM $CaCl_2$ substantially altered the aggregation pattern. According to Caillard et al. (2009), the presence of divalent cations Ca^{2+} promotes electrostatic screening and facilitates ionic bridging across the negatively charged carboxylate groups on the oat protein chains. By neutralising surface charges, Ca^{2+} eliminates the electrostatic repulsion that keeps proteins dispersed. Consequently, the system becomes pre-aggregated with compact, isolated aggregates. Their study confirmed that such salt-induced protein clusters become highly compact and less porous.

This supports the hypothesis that the calcium concentration induced pre-aggregation prior to fermentation, thus reducing protein solubility. The steric hindrance prevented a continuous, water-trapping gel matrix during subsequent acidification. Furthermore, a complex food matrix such as OPC, where both protein and starch form continuous networks, starch gelatinisation must occur prior to protein aggregation. Otherwise, the development of a continuous starch phase is hindered (Johansson 2024). Consequently, as the $CaCl_2$ induced protein aggregation prior to heating, the gelatinisation of starch did not occur, thus it

explains why the calcium-fortified yoghurts remained in a low viscosity, fluid state and exhibited lower viscosity than the control Oat.

4.6 Syneresis and Product Development

Water phase separation, or post-fermentation syneresis, was observed in all samples during refrigeration storage. This phenomenon indicates a degree of internal gel network contraction or an insufficient WHC. The observed syneresis was reversible upon mechanical agitation, which points to a lack of attractive forces between the phase-separated structural components. This weak interaction hypothesis is directly supported by the microstructural arrangements in the CLSM observations (Figure 9 and Figure 12).

The occurrence of macroscopic layer separation correlates with the inhomogeneous structures. These findings are in line with Urbonaite et al. (2015) who stated that an uneven distribution of solid particles within a fluid matrix is a decisive factor driving low WHC and weak structural cohesiveness. Accordingly, this interpretation is further supported by the quantitative results from the primary WHC and WSI testing assays (Section 4.2).

From a product development standpoint, mitigating this structural syneresis requires an enhanced phase-filling effect. This could be done by incorporating dietary fibres, lipids or complex carbohydrates to reduce serum mobility (Brückner-Gühmann et al. 2019a). For example, a study by Dhakal et al. (2025) on lupin-oat yoghurt alternatives demonstrated that by incorporating 2% lupin protein increased WHC by 38-94%, thereby reducing syneresis in the plant yoghurt analogue. Consequently, an additional increase beyond the 25% PPI inclusion, explored in this study, could serve as a baseline for future studies mapping the impact of protein density and molecular functionality on moisture retention and plant gel structure.

4.7 Summary of Results and Interpretations

The results summarised in Table 4 of the physicochemical properties show that ultrasonication reduced the particle size and increased solubility, which improved interfacial properties such as foaming and emulsifying capacity. The inclusion of PPI further enhanced these effects, particularly at 25% inclusion, where the highest foaming and emulsifying capacities were observed. The measured WHC appeared to be lowered by ultrasonication, however this was likely due to incomplete sedimentation due to the increased solubility rather than the actual hydration

properties. Collectively, the results indicate that the combination of ultrasonication and PPI incorporation improved the functional performance of the OPC system.

Table 4 Summary of physicochemical properties results and mechanism behind it.

Analysis	Main results	Mechanism behind result
Microstructure	Ultrasonication reduced particle size and produced a more dispersed matrix	Acoustic cavitation disrupted protein clusters.
WSI	U-samples showed higher WSI than C-samples.	Reduced particle size and disruption of complexes increased surface area and improved solubility.
WHC	C-samples showed higher apparent WHC than U-samples.	Increased solubility in U-samples prevented pelleting after centrifugation, likely underestimating WHC values.
FC	Ultrasonication increased FC, particularly in U-Pea25. Control samples showed low FC.	Increased solubility and smaller particles improved protein migration to the interface-
FS	No FS across all samples, except for U-Pea25-	Higher PPI content together with sonication likely promoted formation of a more elastic interfacial film.
EC	Ultrasonication and increased percentage of PPI improved EC. Highest observed in U-Pea25	Particle size reduction and exposure of hydrophobic regions possibly improved adsorption at interface.

A critical finding of this study was the rheological contrast between the fermented samples (Table 5). A slight increase in shear stress and yield stress was observed in all the yoghurts compared to the milks. Overall, the rheological and microstructural

analyses consistently demonstrated that supplementation with PPI substantially improved gel formation in fermented OPC systems.

In contrast, the $CaCl_2$ addition alone did not promote gel matrix formation under the tested circumstances. CLSM observations supported the rheological findings, where Pea25 exhibited a dense continuous matrix, associated with high yield stress and viscosity, whereas oat-only and calcium-fortified yoghurt systems displayed a discontinuous, aggregated structure and did not transform into a gel upon fermentation.

Table 5 Summary of sample formulation results in milk and yoghurt systems and the primary mechanisms.

Formulation	Neutral pH (milk), M-samples	Acidic pH (yoghurt), Y-samples
OPC	**High viscosity:** Thickness likely provided by beta-glucan and gelatinised starch	**Liquid phase:** No gel structure, moderate increase in resistance
OPC+PPI	**Decreased viscosity of OPC:** possible electrostatic repulsion from pea proteins	**Gel structure:** Acid induced protein gelation at pI. (PPI 25%)
OPC+$CaCl_2$	**Decreased viscosity of OPC:** possible electrostatic screening by Ca^{2+} neutralise negative surface charge of proteins, partial aggregation	**Liquid phase:** Possible pre-aggregation (salting out), prevents network formation

5. Conclusion

Formulating an oat-based yoghurt analogue using OPC, PPI, $CaCl_2$ and ultrasonication profoundly reshaped both the fluid characteristics and the final fermented structure. Ultrasonication reduced matrix particle size and increased solubility, which improved both foaming and emulsifying capacities. At neutral pH, PPI inclusion initially decreased oat milk viscosity, but upon fermentation, the 75:25 (OPC:PPI) formulation was the only sample that successfully transitioned into an acid-induced gel with a distinct yield stress of approximately 24 Pa.

Conversely, $CaCl_2$ fortification induced preaggregation (salting-out), which hindered continuous network formation and actively weakened the final yoghurt structure. Despite overall post-fermentation syneresis, the texturally enhanced Pea-blended gel matrix demonstrated that incorporating a threshold concentration of 25% PPI combined with acoustic sonication represents a viable processing strategy to build structural integrity from the used OPC base.

Further studies could investigate how stabilising hydrocolloids could minimise phase separation and evaluate a pure PPI yoghurt to conclude whether the PPI inclusion with OPC is synergistic, or if the PPI itself is the functionality-improving mechanism. Additionally, it would be interesting to know how the viscosity would be affected if $CaCl_2$ is added after starch gelatinisation. Optimising these matrix dynamics will help elevate sensory and application qualities of plant-based dairy complements while expanding commercial pathways for domestic Swedish crops.

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Popular science summary

If you are looking to complement your diet with some more plant-based foods, you naturally expect them to match your sensory experiences of the dairy and meat products you already enjoy. For instance, you expect a yoghurt, whether plant or dairy based, to have a creamy, smooth and viscous texture. But what about nutrition? The secret to a highly nutritious plant-based yoghurt lies in combining cereal and pulse to achieve a complete amino acid profile.

A recent study evaluated the structural development of an oat-based yoghurt to see how this texture can be accomplished. By using ultrasonication, a clean technology that uses high-intensity sound waves, particle size can be reduced to produce a fine, uniform food matrix. This improved the solubility, and emulsion properties.

One yoghurt that was formulated with oat and pea protein at a 25:75 (pea:oat) ratio resulted in a yoghurt like texture. However, the other yoghurts with less or no pea, or calcium added in different concentrations, stayed liquid and did not settle into a thicker texture. Another yoghurt test with oat and added calcium showed that the viscosity decreased of both the oat milk and yoghurt analogues, compared to no added calcium. This was due to the calcium making the protein clusters neutral which caused them to aggregate into large clumps. Therefore, a protein network could not form during fermentation.

After refrigeration, the yoghurts showed phase separation, probably because of a lack in attractional forces or too little filling material in the liquid matrix. Fortunately, this could easily be fixed by stirring the yoghurt for a homogenised texture again. Future development of the yoghurts could focus on reducing this phenomenon by either adding more pea protein or adding a water absorbing agent like dietary fibres, which would lock the liquid phase into place. By understanding the molecular dynamics, food scientists can improve Swedish pant-based nutritious food products.

Populärvetenskaplig sammanfattning

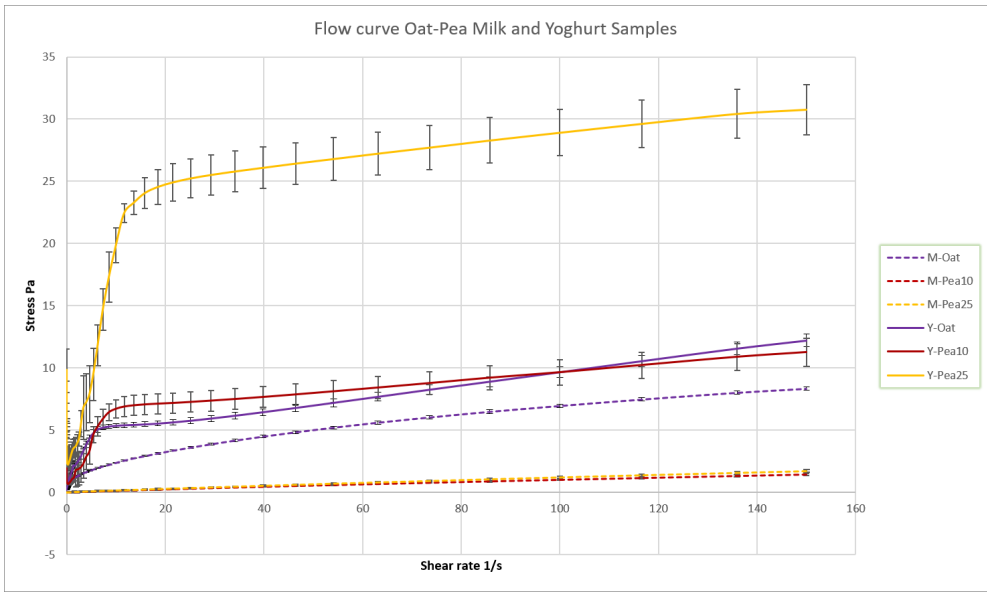
Om du vill komplettera din kost med mer växtbaserad mat förväntar du dig naturligtvis att den ska matcha de sensoriska upplevelserna av de mejeri- och köttprodukter som du redan gillar. Du förväntar dig till exempel att en yoghurt, oavsett om den är växt- eller mejeribaserad, ska ha en krämig, slät och fyllig konsistens. Men hur står det till med näringsinnehållet? Hemligheten bakom en näringsrik växtbaserad yoghurt ligger i att kombinera spannmål och baljväxt för att uppnå en komplett aminosyraprofil.

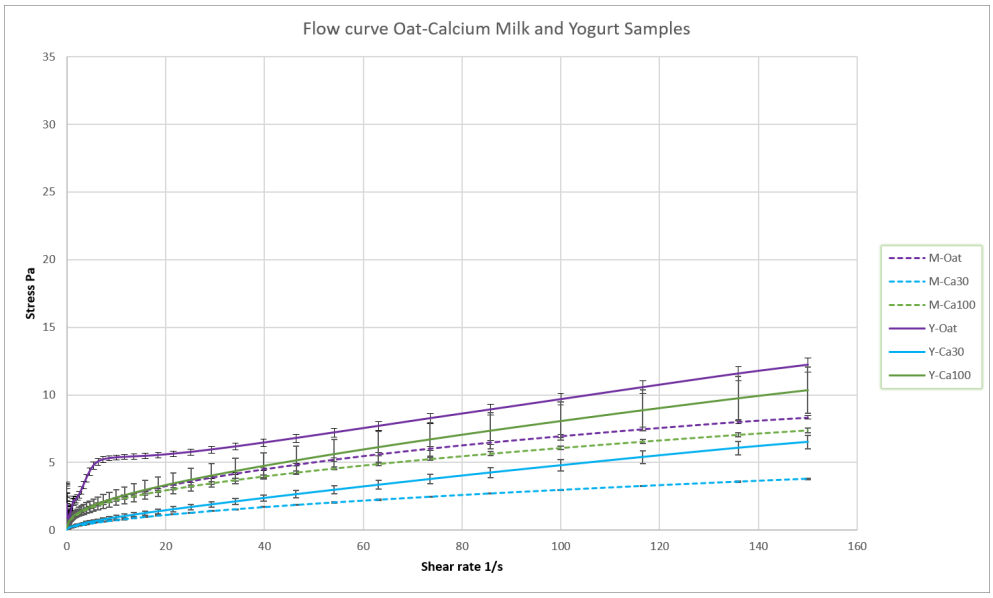
I en nyligen genomförd studie utvärderades den strukturella utvecklingen hos en havrebaserad yoghurt för att se hur en idealisk konsistens kan uppnås. Genom att använda ultraljudsbehandling som är en ren teknologi som använder högintensiva ljudvågor, så kan råmaterialets partikelstorlek minskas för att skapa en jämn mikroskopisk struktur. Denna process förbättrar både proteinernas löslighet och deras emulgerande egenskaper.

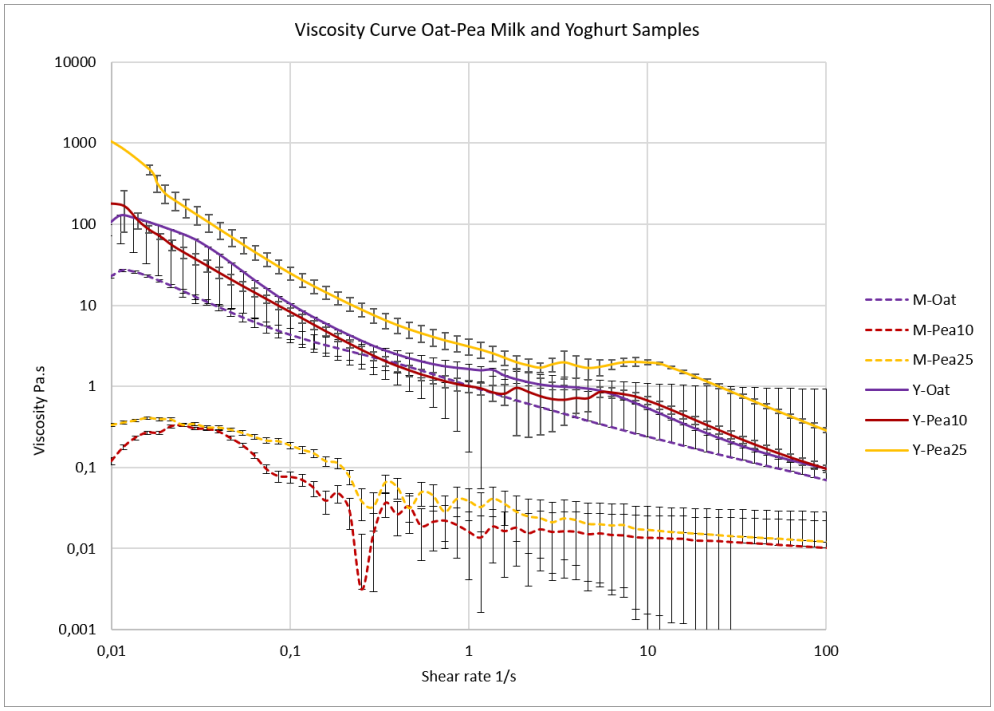
Ett av recepten som kombinerade havre- och ärtprotein i förhållandet 25:75 (ärta till havre) lyckades få en tjock, yoghurtliknande konsistens. De andra recept-formuleringarna som innehöll mindre eller inget ärtprotein, eller olika koncentrationer av tillsatt kalcium, förblev dock flytande. Intressant nog visade tester att tillsats av kalcium faktiskt minskade viskositeten hos både den ursprungliga havremjölken och de färdiga yoghurtanalogerna jämfört med kontrollen utan kalcium. Detta berodde på att kalciumet neutraliserade de elektriska laddningarna hos proteinerna. Det fick dem att klumpa ihop sig i stora, isolerade öar snarare än att bilda ett sammanhängande, tätt nätverk under fermenteringen.

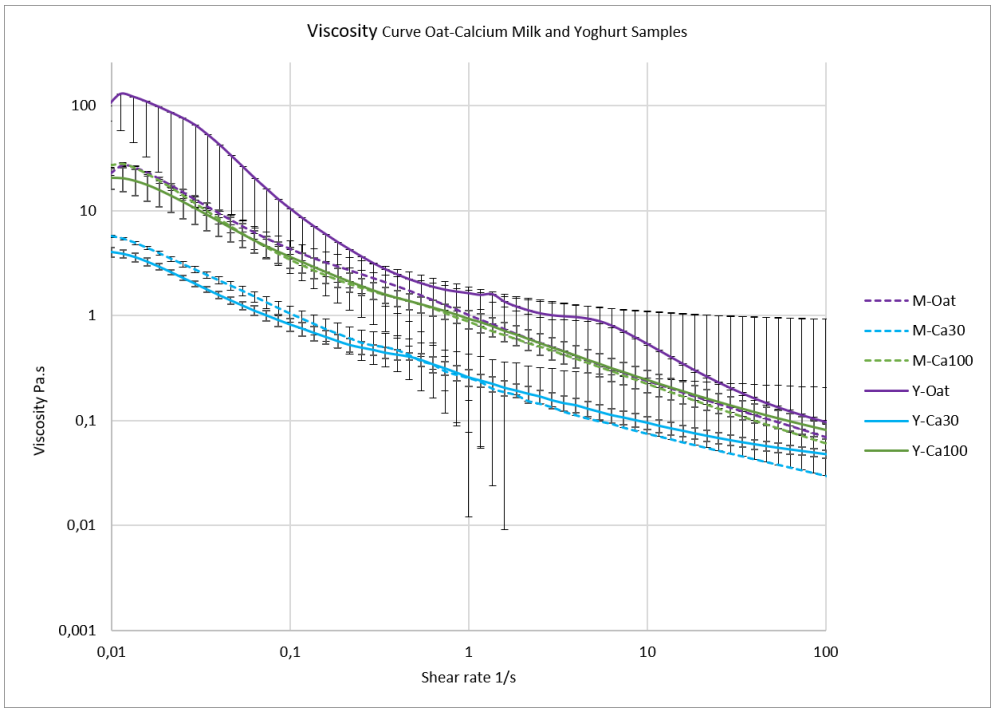
Efter kylning uppvisade alla sorters yoghurt fasseparation, där vätska frigörs från gelen, troligtvis på grund av för svaga krafter mellan partiklarna och för lite bindande material i vätskan. Lyckligtvis kunde detta enkelt åtgärdas genom att röra om i yoghurten för att återställa en slät och homogen konsistens. Framtida studier skulle kunna fokusera på att minska denna separation, antingen genom att öka andelen ärtprotein eller genom att tillsätta vattenbindande ämnen som kostfibrer för att låsa vätskefasen på plats. Genom att förstå den molekylära dynamiken kan livsmedelsforskare fortsätta att förbättra kvaliteten på näringsrika, svenska växtbaserade livsmedel.

Appendix 1 Rheological curves with standard deviation






Viscosity Curve Oat-Pea Milk and Yoghurt Samples
10000
1000
100
10
1
0,1
0,01
0,001
Viscosity Pa.s
0,01
0,1
1
10
100
Shear rate 1/s
M-Oat
M-Pea10
M-Pea25
Y-Oat
Y-Pea10
Y-Pea25


Viscosity Curve Oat-Calcium Milk and Yoghurt Samples
100
10
1
0,1
0,01
0,001
Viscosity Pa.s
0,01
0,1
1
10
100
Shear rate 1/s
M-Oat
M-Ca30
M-Ca100
Y-Oat
Y-Ca30
Y-Ca100

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