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Use of cellulose microfibrils and potato protein to form double network gels

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ABSTRACT

The formation of double network hydrogel systems is investigated using cellulose microfibrils from citrus fibre and a thermally gelling potato protein. We study how the system transitions from a single, to a double network gel, as the potato protein network forms a second network entangled within the network of cellulose microfibrils. The system is studied via oscillatory rheology, namely temperature and amplitude sweeps. We find that the contribution of the native potato protein on the single network cellulose microfibril gel is minimal. However, when the protein is thermally denatured, the cellulose microfibrils and gelled protein act synergistically to contribute to the storage modulus of the double network gel. At low protein concentrations, the addition of the cellulose microfibril network reduces the minimum protein concentration for gel formation. At low to moderate protein concentrations, the cellulose network interpenetrates the protein network, significantly increasing the elastic modulus. At high concentrations of protein, the protein gel network entirely dominates the rheological response, though this is observed up to a certain ratio of protein to fibre. We link the observed rheological properties to the microstructure via confocal laser scanning microscopy. Flocs of the cellulose microfibrils are observed with the secondary protein network entangled throughout. These dense flocs are likely to be the key contributor to the increased mechanical properties of the double network system.

1. Introduction

Single network gels have been studied in great detail, and they have found applications in various fields, such as medicine (Hadde et al., 2021), tissue engineering (Dai et al., 2023), pharmaceuticals (Prajapati et al., 2013) and the food industry (Sharma et al., 2023). Single network hydrogels are typically prepared using polysaccharides (Ren & Ai, 2024) or proteins (Munialo et al., 2018). These materials provide different properties and strengths depending upon their gelation mechanism (Nguyen et al., 2024). For example, some materials display physical cross-linking, i.e., they gel upon physical treatments including temperature, ionic strength, and pH changes (Sharma et al., 2023). Some materials display chemical cross-linking, i.e. they gel upon addition of molecular or ion cross-linkers, enzyme treatment, or direct chemical reactions between polymers (Nguyen et al., 2024). While single component gels offer better control over their assembly and properties, combining multiple gelling agents simultaneously, i.e. mixed gels, allow for even more versatility of strength and functionality. Mixed polysaccharides and/or proteins have also been widely studied in the food industry (de Jong et al., 2009). In the field of mixed gels, remarkable new gel structures built from interpenetrating networks, where each network contributes differently have recently emerged (Li et al., 2020; Niu et al., 2019). Such double network (DN) gels can display improved hardness, strength, and toughness compared to single network gels (Gong, 2010). Previous works have found that the networks formed in DN gels can act synergistically, providing higher firmness than each component alone (Gong, 2010). Among others, DN gels have been generated using combinations of two distinct polymers (Gou et al., 2021), particle-polymer (Jiang et al., 2022), particles-protein (Gouzy et al., 2019; Peng et al., 2019), and particle-particle (Macias-Rodriguez & Velikov, 2022) combinations.

Cellulose microfibrils (CMF) are a particularly interesting material for variety of reasons (Sanchez-Salvador et al., 2023; Xu et al., 2024). CMF are useful from a sustainability perspective, as cellulose is the most abundant biopolymer on the planet and can be recovered from the waste streams of other manufacturing processes (Costa et al., 2018; Wallecan

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et al., 2015). Furthermore, the sources of CMF are a dietary fibre, a non-fermentable material that is beneficial for human digestion (Mudgil & Barak, 2013), and can be used to develop low-calorie foods. Finally, CMF can act as a thickener, as it forms a gel structure when dispersed in water. CMF have a width of 3–4 nm, and are attracted to one another to form fibril aggregates which are approximately 10–25 nm in diameter (Gibson, 2012). This attraction is mediated by hydrogen bonds and van der Waals interactions (Ohshima, 2014; Veen et al., 2014). The fibrils can be temporarily deagglomerated using high shear, but the attractive forces cause the fibrils to reagglomerate, which can lead to gel formation if the agglomeration forms a space-filling network (Kuijk et al., 2013). For these reasons, CMF have been used in various applications, including hydrogels (Gouzy et al., 2019; Peng et al., 2019), emulsions (Dai et al., 2023; Nomena et al., 2021), and edible films (Valencia et al., 2019).

A potato protein isolate rich in patatin is used to form the second network, as it is a thermally gelling protein, and has a fairly low denaturation temperature, 66.6 °C (Schmidt et al., 2019; Tan et al., 2023). Patatin is a globular protein and has a molecular weight range of 45–50 kDa (Katzav et al., 2020). When heated in aqueous solutions, the potato proteins unfold to expose hydrophobic regions, which allows for aggregation of the protein. If the concentration of protein is high enough, this leads to irreversible gelation and network formation (Andlinger et al., 2021; Phillips & Williams, 2011). Potato protein is abundant, non-allergenic, and contains 18 different amino acids, including all 9 essential amino acids (Beals, 2018), making it a complete protein with high nutritional value (Hu et al., 2024; Kaldy, 1972; Katzav et al., 2020). These benefits make potato protein a particularly attractive option for use in foods. Patatin has an isoelectric point of 4.9 (Waglay & Karboune, 2016). The range of mechanical strengths achievable via protein gel systems alone is limited, as a minimum concentration is required to achieve network formation, as mentioned above. At the upper limit of protein concentration, the solubility of the protein is reduced (Tang et al., 2022).

Recent studies indicate a rapidly growing attention to plant protein-cellulose fibrils mixed gels (Ryu & McClements, 2025; Wang et al., 2025). Incorporating cellulose fibrils in high potato protein (fixed at 20 wt%) gels appears to be more effective at modulating the texture of the hydrogels than incorporating cellulose nanocrystals (Ryu & McClements, 2025). First insight into the mechanism of heat-induced gelation improved in soybean protein isolate-bacterial cellulose mixed gels has only recently been revealed (Wang et al., 2025). However, the formation of DN gels of potato protein and cellulose microfibrils, and the concentration dependent contribution of both to the microstructure and rheology of gel is not fully understood. The purpose of this study is to better understand the tuneable rheology of the gel, before heating via CMF, and after heating, via the potato protein. As each network within DN gels are known to act synergistically, it may well be the case that CMF allows for less protein to be used, and for the same storage modulus to be obtained. This could lead to a cost and calorie reduction. As the contribution of CMF and potato protein to the structure and rheology of DN gels is better understood, this understanding could be used to develop novel and complex food systems, such as plant-based emulsions and foams without the use of chemically modified ingredients.

We study the contribution of both potato protein and CMF to the rheology of the gel by preparing samples with varying concentrations of both materials, analysing them via oscillatory rheology, namely temperature and amplitude sweeps, and linking rheological behaviour with sample microstructure via Confocal Laser Scanning Microscopy. We find that CMF and potato protein can contribute synergistically to the structure and storage modulus of the gel; however, this is concentration dependent.

2. Methods and materials

2.1. Materials

To prepare the dispersions, citrus fiber Herbacel AQ + type N (65 wt % of cellulose from primary plant cell walls, 3.7 wt % of hemicellulose, and 5 wt% of proteinaceous materials (Fechner et al., 2013)) from Herbafood Ingredients GmbH, Germany was used as a source of cellulose microfibrils. Potato protein isolate Solanic 200 (according to supplier specification: 90 wt% total protein, 0.2 wt% dietary fibre, 0.1 wt% lipids, 0.2 wt% carbohydrates) was obtained from Avebe. Both materials were dispersed in demineralised water. Sodium Hydroxide pellets were received from J.T. Baker, and used to prepare a 5 wt% NaOH solution, which was used to neutralize the pH each of the samples. Antibacterial agent Proxel GXL, provided by Arxada, was also added to the samples. Calcoflour White and Rhodamine-B, obtained from Sigma Aldrich were used as flourecent dyes for the Confocal Laser Scanning Microscopy.

2.2. Sample preparation

Solutions were prepared by dispersing varying amounts of potato protein (range of 0–10 wt%) and citrus fibre (0–2 wt%) in demineralised water. The dispersions were prepared by firstly dispersing citrus fibre in demineralised water using a magnetic stirrer, at 500 rpm for 10 min. The dispersion of citrus fibre typically had a pH of 4, which is below the isoelectric point of the potato protein (Katzav et al., 2020). The pH of the protein in water is approximately 7, and so the pH of the fibre dispersion was neutralised using NaOH solution before the protein is added. This was to avoid passing the protein through its isoelectric point, which causes it to aggregate. Next, the potato protein was added to the dispersion, again by dispersing at 500 rpm for 10 min with a magnetic stirrer. This mix was then homogenised further using a high shear laboratory mixer (Silverson L5M, USA) at 2000 rpm, using a screen with 1 mm holes, for 1 min. Low shear mixing and a short mixing time were employed to avoid incorporating air into the samples. The sample vessel was moved by hand around the head of the mixer to ensure that the dispersion was mixed as homogenously as possible. Finally, the dispersion was passed through a Microfluidizer (Microfluidizer M110-S, USA) with a 200 μm diameter, Z-shape, ceramic channel, operating at a pressure of 1000 bar, the microfibrillar network structure of the cellulose is displayed in Supplementary Note 1, and the drastic increase in dispersion elasticity measured before and after microfluidisation of the cellulose is displayed in Supplementary Note 2. Passing these samples through the Microfluidizer was found not to affect the thermal denaturation behaviour of the potato protein, while the presence of the citrus fibre seemingly did (see Supplementary Note 3). The cooling coil of the microfluidizer was submerged in ice water to avoid overheating the samples, the temperature of the samples was limited to the range 19–30 °C. Next, a small amount of antimicrobial agent, approximately 85 mg per gram of sample, was added to the dispersions. Finally, the pH of the dispersions was checked and adjusted to 7 with the NaOH solution, if necessary. Images of samples can be found in Supplementary Note 4.

2.3. Oscillatory rheology

Oscillatory rheology measurements were completed on each of the samples. Each measurement was completed using a stress-controlled rheometer (MCR 302, Anton Paar, Austria). A sandblasted, plate-plate geometry with a 50 mm diameter was used, with a 1 mm gap. Mineral oil (Light White, Amresco) was applied over the exposed edges between the plates, and a solvent trap set-up was used, both of which were done to avoid sample drying. A water bath (Viscotherm VT2, Switzerland) was used, along with a Peltier system to control the temperature of the set-up. Temperature sweeps were performed on the samples to analyse how the flow properties changed during denaturation of the protein, and the samples were heated from 20 °C to 80 °C, held at 80 °C for 5 min, and

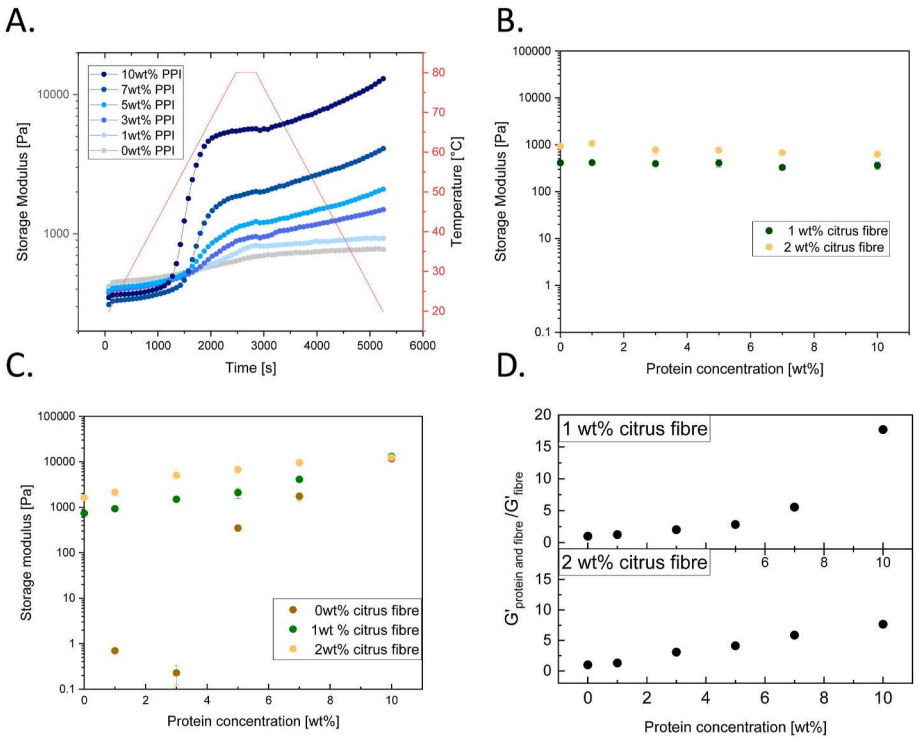

Fig. 1. A) Temperature sweep data for samples with increasing concentrations of potato protein and 1 wt% citrus fibre, at a strain of 0.2 % and frequency of 1 rad/s. B) The storage modulus of the dispersions at 20 °C prior to heating, plotted against potato protein concentration. C) The storage modulus of the dispersions at 20 °C after heating, plotted against potato protein concentration. D) Amplification factor, i.e. storage modulus of the DN gel at varying concentrations of protein divided by the concentration of the initial single network.

subsequently cooled to 20 °C. The amplitude of the oscillations was kept constant at 0.2 %, as it is a value within the linear viscoelastic regime for all samples prepared. The oscillation frequency used was 1 rad/s. The rate of heating was 1.5 °C/min. Amplitude sweeps were conducted on samples before and after heating, to determine the linear viscoelastic regime for the samples, and identify the appropriate amplitude to use during the temperature sweeps. The amplitudes used ranged from 0.01 to 200 % strain, again at a constant frequency of 1 rad/s, an example of which can be found in Supplementary Note 5. All measurements were completed at least in duplicate.

2.4. Confocal scanning laser microscopy (CSLM)

Microstructure images were taken using a confocal microscope (Zeiss LSM 880 Germany), using both a 20 × and 63 × magnification. The samples were dyed by adding Rhodamine B (518 μL/g of sample) and Calcofluor White (64 μL/g of sample). The Rhodamine B dye was excited at wavelength 531–703 nm and the Calcofluor White was excited at wavelength range of 410–509 nm. The laser used to excite the Rhodamine B was 405 nm, and the laser used for the Calcofluor White was 514 nm. A sequential imaging protocol was used to avoid crosstalk between channels. The samples were placed into Coverwell imaging chambers (Grace BioLabs, United Sates) and then imaged. Each chamber was sealed in a waterproof case and heated in a water bath at 80 °C for 30 min to denature the protein, trigger aggregation and, if the protein concentration is high enough, allow it to form a network. The samples were then cooled in ice water for 5 min and imaged once again, using the same protocol described above.

3. Results and discussion

3.1. Rheology of CMF and potato protein dispersions

To study the effect of potato protein concentration, samples were prepared with protein concentration ranging from 0 up to 10 wt%, at two different concentrations of citrus fibre, 1 and 2 wt%. Above 2 wt% of citrus fibre, blockage of the Microfluidizer is known to occur, but was not observed for the samples prepared here. Temperature sweeps were performed on each of the dispersions, an example of the data obtained is displayed (Fig. 1A).

Firstly, we consider the single network, CMF system, and how it is affected by the presence of native potato protein, i.e. before heating and gelation of the protein. Amplitude sweeps for dispersions of increasing concentration of Citrus Fibre in water are displayed in Supplementary Note 6 and demonstrate that all samples behave as rheological gels (G' > G"). Given that the native protein does not form a network, it is expected that the CMF concentration will be the dominant contributor to the storage modulus of the gels, and indeed that is what is observed. In Fig. 1B, the dependence of the storage modulus of these gels at 20 °C prior to heating, on protein concentration is displayed. There is seemingly little effect of potato protein on the storage modulus of the gels.

During the heating period of the temperature sweep, there is an increase in the storage modulus of all samples that have potato protein present (Fig. 1A). This is similar to behaviour observed in previous works studying gels of potato protein alone, i.e. no other structurant (Zhou et al., 2023), and in emulsions (Domian et al., 2023). This increase in storage modulus is due to aggregation of the protein, which leads to network formation. In the case of patatin, the main protein present in Solanic-200, aggregation is driven by attraction between hydrophobic regions of the protein, which are exposed during the heating and denaturation process (Andlinger et al., 2021; Li et al., 2024). The storage modulus continues to increase as the samples are cooled back to 20 °C, as hydrogen bonding and van der Waals interactions of the protein network strengthen (Ould Eleya et al., 2004).

To understand the contribution of protein on the elasticity of the DN structure, the storage moduli of samples at 20 °C, after heating and subsequent cooling, are plotted against protein concentration, for dispersions with 0, 1, and 2 wt% citrus fibre (Fig. 1C). An amplification

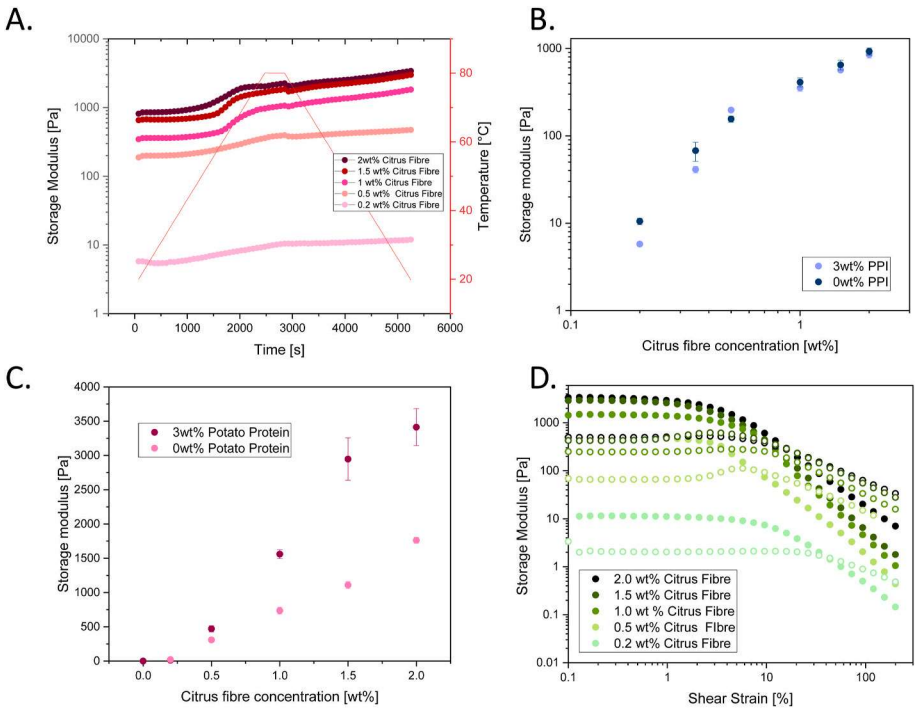

Fig. 2. A) The effect of increasing temperature on dispersions of potato protein and increasing concentrations of citrus fibre, at a strain of 0.2 % and frequency of 1 rad/s. For readability, not all measurements are displayed B) The storage modulus of the dispersions prior to heating, plotted against the citrus fibre concentration. Samples with and without protein present. C) Scaling of the storage modulus of the double network i.e. after heating, plotted with citrus fibre concentration. D) Amplitude sweeps for samples of increasing citrus fibre concentration with 3 wt% potato protein, after heating.

factor, given by $G'_{protein\ and\ fibre}/G'_{fibre}$ ratio is plotted versus protein concentration, at fixed concentration of fibre (Fig. 1D). For all concentrations of CMF, the storage modulus increases with potato protein concentration, as this allows for more interactions between protein in the network, and thus a denser structure. We observe a significant increase in the storage modulus of samples with pure potato protein (i.e. no CMF) when the concentration increases from 3 to 5 wt% (Fig. 1C). This suggests there is a critical concentration of potato protein, below which the aggregated protein cannot form a network structure. This has been observed for globular proteins in general (Schmitt et al., 2019). One study found this minimum concentration to be 6 wt% of patatin (Creusot et al., 2011), though this was for lab-extracted patatin, rather than commercial patatin, as used in our study.

As the protein concentration increases from 0 to 3 wt%, for samples of 1 wt% and 2 wt% citrus fibre, the storage modulus also gradually increases. This demonstrates that the protein here can contribute to the existing CMF network at very low concentration. This is likely because the CMF network restricts sedimentation of particles (Nordenstrom et al., 2022), which in this case is the aggregated protein, meaning it can form a network, but only within the flocs of the cellulose microfibrils. However, it is not able to form a network in areas which are void of the cellulose. The yield stress behaviour of citrus fibre dispersions (Serial et al., 2021) as well as that of CMF dispersions more generally (Nechyporchuk et al., 2016) has been previously discussed. For the concentrations of CMF studied here, the concentration required for the protein to begin contributing to the storage modulus is shifted to below 1 wt% of protein. It may be that rather than forming space-filling networks within the CMF, the protein forms discrete aggregates which also contribute to the storage modulus. We use confocal microscope later in the study to elucidate the mechanism by which the storage modulus increases at low concentrations of protein when CMF is present. Notably, the storage modulus of the samples is initially higher when there is more citrus fibre present. This demonstrates that, at these concentrations, the CMF and the potato protein contribute synergistically to the elasticity of the system. At 10 wt% potato protein, the storage modulus is similar for all concentrations of citrus fibre, suggesting that the contribution of the CMF to the storage modulus of the system is negligible, and the storage modulus is entirely dominated by the gelled protein network. A similar phenomenon has been observed previously, where the synergistic contribution to the storage modulus from bacterial CMF and whey protein isolate is very little, in comparison with protein alone (Peng et al., 2019).

To investigate the contribution of the CMF to the system, dispersions with a constant amount of protein, 3 wt%, and increasing concentrations of CMF, ranging from 0 to 2 wt% were studied. This quantity of protein was chosen as it is below the critical concentration of protein for gelation, as discussed above, and illustrated in (Fig. 1C). This concentration range of CMF was selected as pre-experiments demonstrated that it delivered both pourable and spoonable textures.

The samples were analysed using the same temperature sweep method as detailed above, and an example of the results, at 3 wt% protein, are displayed (Fig. 2A). Before the samples are heated, the scaling of storage modulus with citrus fibre concentration, with and without protein present remains similar (Fig. 2B), suggesting once again that the native protein has very little effect on the microstructure and interactions of the CMF within the gel.

Then, the contribution of the CMF to the DN gel was studied, by comparing the scaling of the storage moduli of the samples versus concentration of citrus fibre, for samples with and without potato protein present, at 20 °C after heating and cooling (Fig. 2C). Gels with 3 wt % potato protein and no fibre, as displayed in Fig. 1C, had a storage modulus of 0.23 Pa, as the protein concentration is not high enough for the protein to form a space filling network. A notable observation is that with as little as 0.5 wt% citrus fibre, the storage modulus of the system with protein increases drastically, compared to the sample with no protein present. This trend remains for higher concentrations of CMF, and this further demonstrates that the protein and the CMFs act synergistically to increase the elasticity of DN gels.

To further understand this DN system, amplitude sweeps were

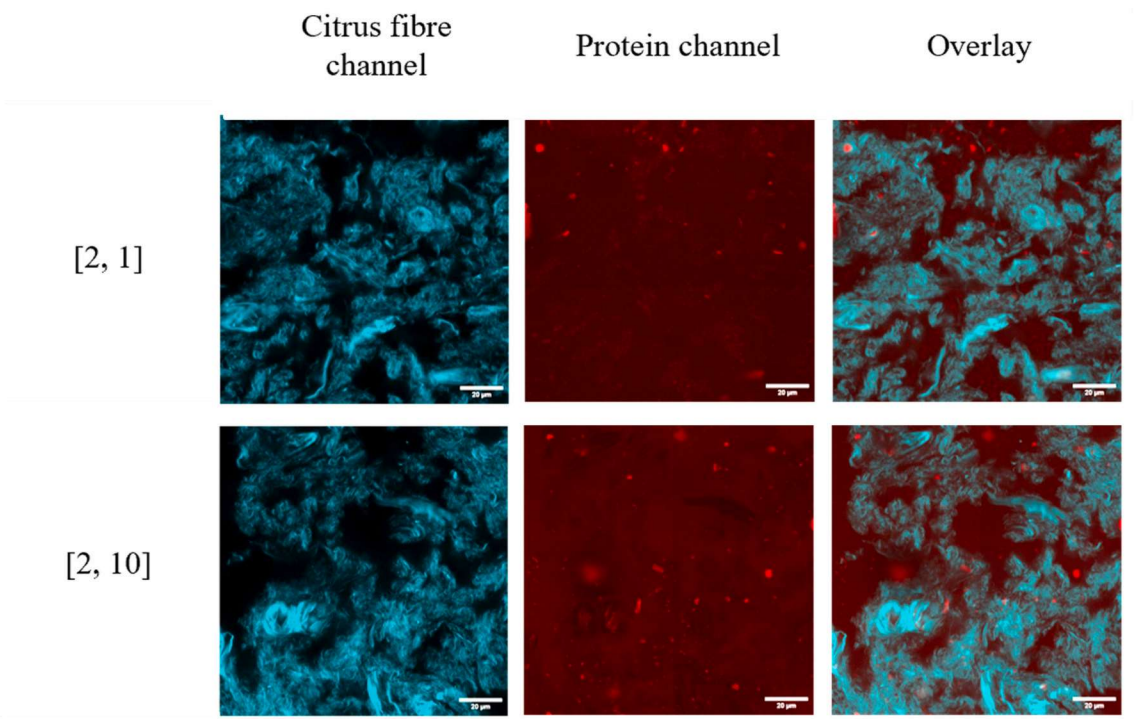

Fig. 3. *CSLM images taken of samples of constant citrus fibre concentration and potato protein concentration, unheated samples. Scale bar is* 20 μm*, concentration (wt%) of each component given by* [Citrus fibre, Potato protein]. *Readers are referred to the online version of the article for better viewing of the images, and Supplementary Note 8 for the full set of images.*

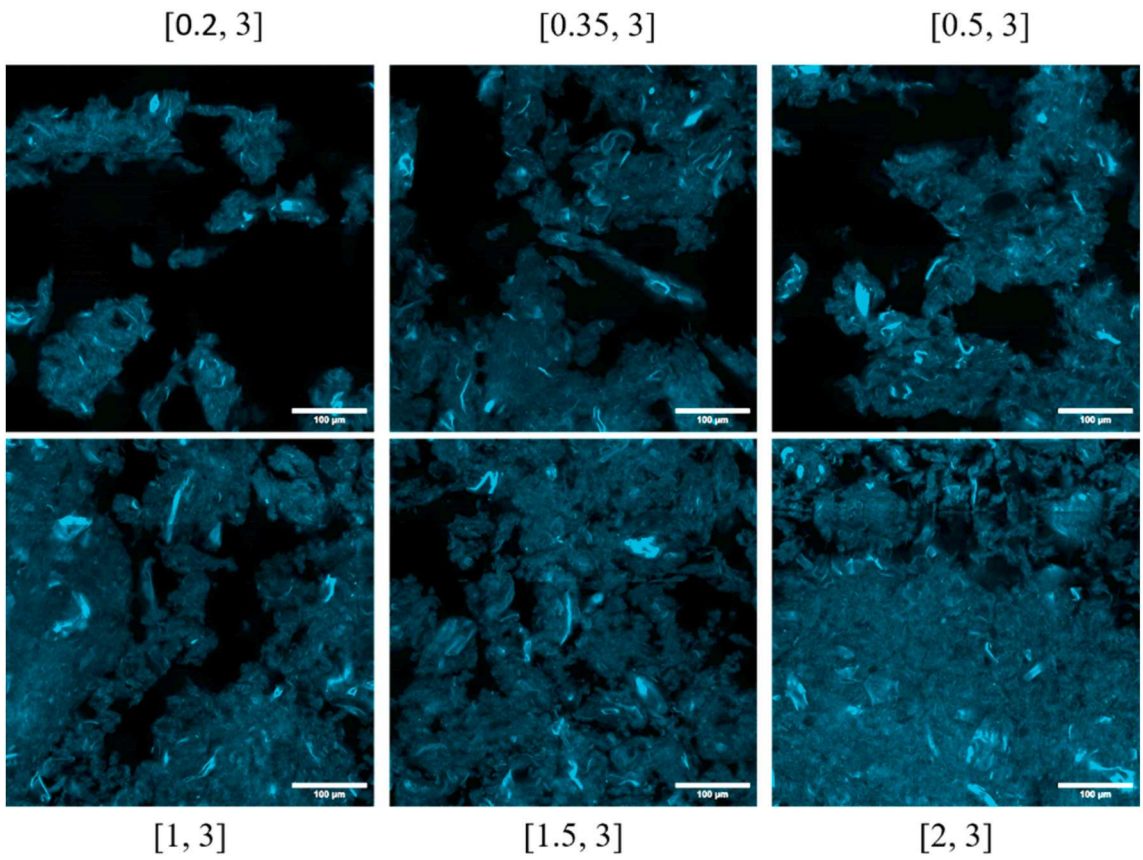

Fig. 4. *CSLM images taken of samples of constant potato concentration and increasing citrus fibre concentration, unheated samples. Scale bar is* 100 μm*, concentration (wt%) of each component given by* [Citrus fibre, Potato protein]. *Protein channel not displayed. Readers are referred to the online version of the article for better viewing of the images.*

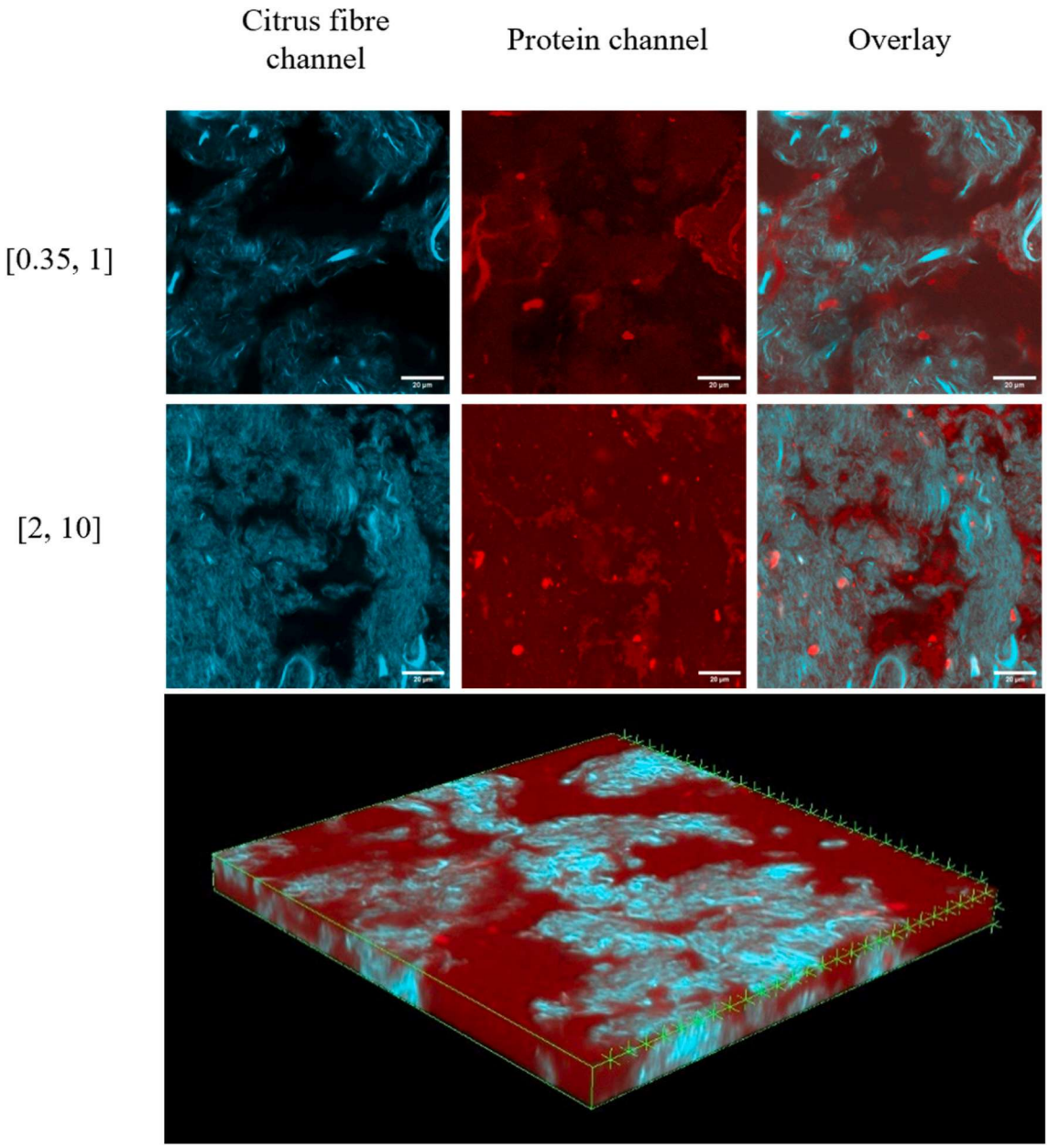

Fig. 5. *CSLM images taken of samples with low and high concentrations of citrus fibre and potato protein. Scale bar is* 20 μm*, concentration (wt%) of each component given by* [Citrus fibre, Potato protein] *(top). Z-stack of sample with 2 wt% citrus fibre, and 10 wt% protein, image taken at 20× magnification (bottom). Readers are referred to the online version of the article for better viewing of the images.*

performed (Fig. 2D).,The storage modulus in the linear viscoelastic regime trended with increased concentrations of citrus fibre and is in keeping with previous studies on the rheology of citrus fibre suspensions (Serial et al., 2021) and cellulose nanofibril suspensions more generally (Nechyporchuk et al., 2016). Each of the samples demonstrated qualitatively similar yielding behaviour, all yielding in the range of 3–10 % strain. Given that there are two different materials providing structure to the gel, both with different yielding mechanisms, one may expect the storage modulus to yield in two stages, however this is not observed in the concertation range studied (see Supplementary Note 7). The likely reason for this is that the CMF allows for the DN gel to be more ductile, i.e. the linear viscoelastic regime extends to a higher amplitude, such that both networks yield together. It may be the case, however, that the brittle protein network does breakdown, but isn't observed via amplitude sweep as it is masked by the strain-weakening flow behaviour of the CMF-protein composite network.

3.2. Structure formation in CMF and potato protein mix gels

To better understand the rheological phenomena discussed above, images of sample microstructure were taken using a confocal scanning laser microscope (CSLM), for samples with increasing concentrations of potato protein and constant concentrations of citrus fibre, and vice-versa. For readability, most of the confocal images have been omitted, but can be found in Supplementary Note 8.

The structure of these gels prior to the formation of the secondary protein network was then imaged, to observe any effect of increasing protein concentration on the CMF gel (Fig. 3). Qualitatively, the size and density of CMF flocs are indistinguishable from each other at the length scale probed via CSLM, as is expected given the lack of variation in their rheological properties. One observable difference between samples is the presence of more protein aggregates with increasing protein concentration, as less of the protein is in solution, however this seemingly

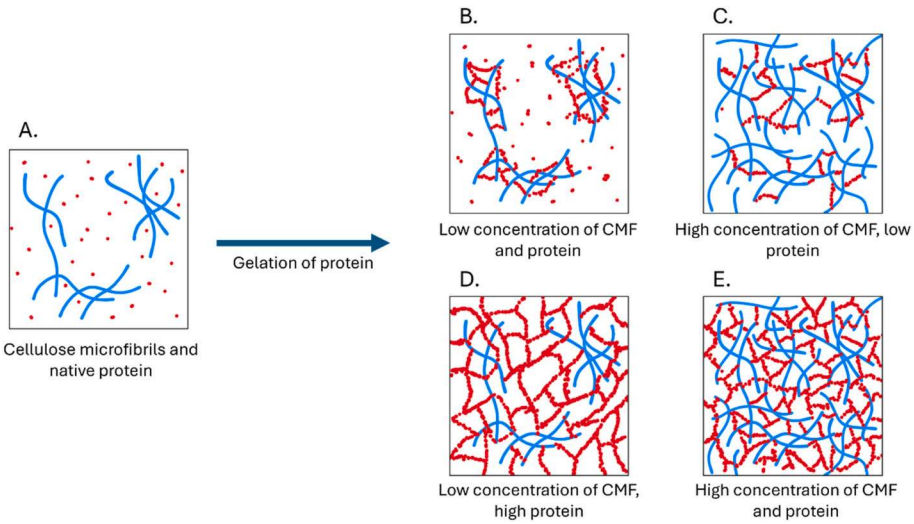

Fig. 6. Illustration demonstrating the proposed mechanism for the formation of the DN gel at varying concentration of CMF (blue) and potato protein (red).

has a negligible effect on the storage modulus of the gels.

Next, samples with increasing amounts of citrus fibre, and constant concentrations of potato protein were imaged. The samples with 3 wt% native protein are displayed in Fig. 4. As expected, qualitative observation indicates that the size and the number of voids within samples decreases with increasing CMF concentration, and, accordingly, flocs of CMF appear more homogenously distributed and interconnected. The formation of flocs is illustrative of the attractive nature of the CMF in this system.

The samples were then heated in a water bath at 80 °C for 30 min, cooled, and imaged again. In Fig. 5 (top), we present confocal images of a sample at low concentration of both CMF and protein, with which the formation of the DN can be best illustrated. At this concentration, there are regions in which the protein network has begun to form inside of, and around flocs of the CMF. In regions where there is no CMF present, however, no protein network has formed. Thus, demonstrating that the CMF network supports the formation of the protein network, likely by restricting sedimentation of protein aggregates during gelation, and that the two networks are at least sterically entangled. As these mixed regions of CMF and protein occupy a relatively small volume of the sample, however, the storage modulus remains low (see Fig. 1C). As the concentration of both CMF and protein increases, these mixed regions then begin to occupy much more of the space within the sample. Nonetheless, areas lacking in CMF remain, in which the protein forms a homogenous network (Fig. 5 Top). Rheology measurements demonstrated that increasing amounts of CMF, with constant protein concentration, led to a higher storage modulus for these samples, suggesting that the regions of homogeneous protein gel network (i.e. void of CMF) are weaker than the mixed areas where the CMF is dispersed throughout, and reinforces the protein gel. As discussed in section 3.1, as the protein concentration increases, eventually, this becomes the dominating contributor to the storage modulus of the DN gel. This is likely caused by the homogenous regions of the network become increasingly denser.

Based on this microstructural and rheological study, we propose the following mechanism of formation of DN gels in CMF-thermally gelling protein mixtures in the concentration ranges studied (Fig. 6).

- **At low concentrations of both CMF and protein**: the protein aggregates and local network is formed within the CMF flocs. This leads to an increase in the elasticity of the flocs and the overall gel. Outside these flocs, the protein concentration is too low and cannot form a space filling network and form a gel (Fig. 6B).
- **At high concentration of CMF and low concentration of protein**: As the CMF takes up more space within the sample, less voids are observed, there are more regions where the protein aggregation, and network formation is supported against sedimentation (Fig. 6C). This leads to a significant increase in the storage modulus. However, the protein concentration is too low to form a space filling network within the CMF flocs.
- **At low concentration of CMF and high concentration of protein**: the protein can form dense networks within CMF flocs, but also beyond them, in the voids, similar to typical protein gels (Fig. 6D). The elasticity of the system is then dominated by the contribution of the protein. This leads to a high storage modulus, regardless of the CMF concentration.
- **At high concentrations of both CMF and protein**: a dense network of protein forms through an already dense network of CMFs, and in any regions void of CMF as well (Fig. 6E). In this case the gel's properties are dominated by the protein network.

A multiscale analysis is required to confirm the proposed microstructure and process of mixed gel formation for CMF-gelling protein systems, and this will be a scope for future research. It is possible that there is a molecular interaction between the protein and the cellulose microfibrils, specifically H-bonding. However, testing for such an interaction is difficult. Any molecular interaction cannot be observed via imaging techniques, and testing for H-bonding, using urea as a chemical probe, would not be able to differentiate between H-bonding between the cellulose and the protein, and the H-bonding between the cellulose microfibrils alone, or the protein network alone. Finally, The citrus fibre used in this study also contains 22.2 wt% of soluble dietary fibre (Fechner et al., 2013), a large fraction of which is pectin. Amplitude sweeps for samples of 2 wt% citrus fibre (Supplementary Note 2), which had not been passed through the Microfluidizer, showed significantly lower elasticity values compared to the microfluidised sample at 2 wt% citrus fibre. This suggests that the contribution to the rheology of the gel is low in comparison to that of the deagglomerated cellulose microfibrils. Nevertheless, contribution of the pectin to the results here can't be entirely ruled out. Therefore, future studies could use cellulose microfibrils which do not have pectin present, for example using bacterial cellulose rather than citrus fibre.

4. Conclusions

This study demonstrates that double-network hydrogels can be prepared using CMF from citrus fibre, and potato protein. It was found that native potato protein has little to no effect on the storage modulus of single network, CMF hydrogels. It was found that up to 7 wt% protein,

the CMF and the protein contribute synergistically to the storage modulus of the mixed gel. Once the protein concentration reaches 10 wt %, however, the protein is in surplus, and the synergistic contribution is negligible, though the specific concentration of protein at which this occurs is likely dependent on the ratio with citrus fibre. This study also demonstrates that 3 wt% protein, when it forms a network after thermal denaturation, is enough to be able to increase the storage modulus of hydrogels with CMF present, and this effect is amplified as the concentration of the CMF increases. This is despite 3 wt% being lower than the critical concentration of protein required to form a network alone. Via confocal microscopy, we observe regions in which the CMF and potato protein are at least sterically entangled, which increased the storage modulus of the sample as a whole. DN gels provide an option for materials which require versatility of strength, functionality, and thermal responsivity, which is not always possible with single network systems. The system studied here could also be used for reformulation of plant-based gels, foams, and emulsions within the food industry.

CRediT authorship contribution statement

Ieuan Roberts-Harry: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Braulio A. Macias-Rodriguez:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Krassimir P. Velikov:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2025.111958.

Data availability

Data will be made available on request.

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