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Evaluation of Dairy Co-product Containing Composite Solutions for the Formation of Bioplastic Films

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Abstract
Replacement of existing plastics derived from non-renewable and petrochemical sources will require the creation of new environmentally friendly polymers. Natural products such as protein containing waste generated from dairy factories have considerable potential for the development of bioplastics. Casein rich material (referred to as DAF-casein) harvested from dairy wastewater using a dissolved air flotation (DAF) procedure exhibits self-associating properties, and has potential for the formation of bioplastics, but produces brittle films. Inclusion of additional biopolymers such as polysaccharides and other proteins to form composites with the DAF-casein has the potential to improve the physical properties of such films. Three polymers, κ-carrageenan, sodium carboxymethylcellulose (NaCMC) and gelatin, were found to form homogenous solutions with DAF-casein. All three were successful in the formation of composite bioplastic films with improved morphology and water stability in combination with DAF-casein. The incorporation of additional polymers with dairy waste stream DAF-casein has the potential for the production of bioplastics.

Keywords Bioplastic films · Casein · DAF · Rheological properties · Electron microscopy

Introduction

Globally, the demand for plastic-based packaging material was estimated to be 275 million metric tons annually in 2015 [1], with much of this material being utilised for the production of short-term, single-use and disposable packaging products. Growing adversity to petrochemical based plastic use has led to the drive to find alternative sources of environmentally friendly bioplastics. Biopolymers such as zein, casein, whey and soy protein have previously been shown to have potential for forming bioplastic films as a potential biodegradable plastic replacement for traditional petrochemical based plastics [2–5]. Many biopolymer plastics may also disintegrate and biodegrade without the production of any

additional toxic substances or environmentally harmful residues, and not require the use of harsh chemical processing [6].

Bioplastics derived from a coproduct or waste material have the advantage that they are using an already available and sometimes underutilised resource. Additionally, their utilisation mitigates the cost of disposal and reduces environmental effects. One highly abundant and underutilised source of protein material is the casein-rich water generated during the cleaning of dairy factory plants [7, 8]. Methods for the concentration of this high volume and relatively dilute casein-containing material have been developed, including the use of a dissolved air flotation (DAF) process. During the DAF process, a stream of nitrogen bubbles is used to collect suspended particles from the dairy wastewater. The suspended particles are harvested as a sludge, which is further processed to enrich for the protein portion, then dried and stored as a powder and is referred to as DAF-casein. This powder contains a complex mixture of proteins, lipids, minerals, sugars and ash. Although the potential for use of this DAF-casein in the production of biomaterials, and the use of waste casein in general, has been reviewed [9, 10], no specific on-going use applications have been developed to date.

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The self-associating nature of caseins facilitates the formation of cohesive bioplastic films, which has been reported previously using technical grade casein [11–13]. It has also been shown that the incorporation of whey protein isolate in the preparation of a casein-based film can have a positive effect on the mechanical properties of the resultant film compared to casein alone [14]. This indicates that there can be advantages in using materials such as DAF-casein that contain mixtures of proteins, rather than a purified protein alone.

Our previous preliminary characterisation of DAF-casein found that the material possesses altered fundamental properties compared to other previously characterised milk materials such as skim milk powder [15]. Compared to reconstituted skim milk powder, DAF-casein was observed to have an altered hydrophobic profile, increased viscosity in solution and was not amenable to rennet coagulation, despite the protein complement appearing to be similar. In addition, DAF-casein alone possessed the ability to form cohesive bioplastic films, but displayed poor mechanical properties. The current study therefore, aimed to evaluate the addition of other biopolymers that would form a homogenous composite solution consisting of DAF-casein, to enable production of a cohesive bioplastic film with more favourable properties.

Materials and Methods

Materials

DAF-casein powder was acquired from Callaghan Innovation, Lower Hutt, New Zealand, that was produced from dairy DAF sludge from dairy waste water obtained from a local dairy company. The composition of this material was reported by Ryder et al. [10]. Gelatin powder (150 bloom, pig skin) was obtained from BDH Chemicals Ltd. (Poole, England). κ-carrageenan was obtained from Go Native NZ (Christchurch, New Zealand). Sodium carboxymethylcellulose (NaCMC) was obtained from CP Kelco (Aanekoski, Finland). The water used in all experiments was passed through a Milli-Q Reference Water System Production Unit from Millipore Corporation (Billerica, MA, USA), and is referred to as 'MilliQ water'.

Formulation of Solutions

All biopolymer solutions of DAF-casein, gelatin, κ-carrageenan and NaCMC were prepared in Milli-Q water at a concentration of 2% (w/v). DAF-casein solutions were prepared at room temperature and titrated to pH 8.0 with 1 M NaOH, which was previously found to be the optimal condition for solubilisation [15]. Gelatin, NaCMC and κ-carrageenan solutions were heated to approximately 60

°C and stirred constantly until a homogeneous solution was obtained. Biopolymer composites were prepared by combining two biopolymers together at a given ratio and stirring for at least 15 min, or until a homogeneous mixture was obtained. All concentrations are given as a percentage (w/v) of total solids present, unless otherwise stated.

Rheology Testing

Rheological measurements of DAF-casein composites were carried out using a Haake™ rotational rheometer (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a 60 mm 1° titanium cone. The temperature was controlled at 20 ± 0.5 °C. The distance between the cone and plate was set at 52 μm. Samples were allowed to equilibrate for 2 min in the machine prior to testing. The shear rate was increased linearly between 0 and 200 s^{-1} over a period of 300 s, with measurements obtained every 3 s. Formulations were prepared three times with each being measured in triplicate on fresh aliquots each time.

Preparation of DAF-Casein Bioplastic Films

Bioplastic films were produced by pouring a DAF-casein solution or composite solutions into a 150 mm diameter petri dish containing a polytetrafluoroethylene (PTFE) liner. Solutions were allowed to dry overnight in a fume hood with air movement at ambient temperature, or until the film lifted from the PTFE base and could be removed as previously reported [16]. Unless otherwise stated, all solutions prepared for production of films were made up at a concentration of 2% (w/v) total solids and pipetted onto the PTFE liner in order to achieve 6.36 mg solids/cm^2.

SEM Imaging

Scanning electron microscopy (SEM) was performed at the Robinson Institute, Victoria University, Wellington (Lower Hutt, New Zealand). Bioplastic film samples that were adhered to aluminium SEM specimen mounts were held under vacuum for at least 1 h prior to imaging. Imaging was undertaken using an FEI Nova NanoSEM microscope. An immersion lens was used for all imaging with a working distance of 5 mm. A voltage of 1.0 kV was applied to the samples and images were collected with a dwell time of 10 μs and frame time of 9.4 s. A spot size setting of 4.0 was used.

Statistical Analyses

Where appropriate, samples were measured a minimum of three times in triplicate (n = 3). Exceptions to this are noted in the appropriate sections. The reported results are the average of the triplicate measurements ± one standard deviation.
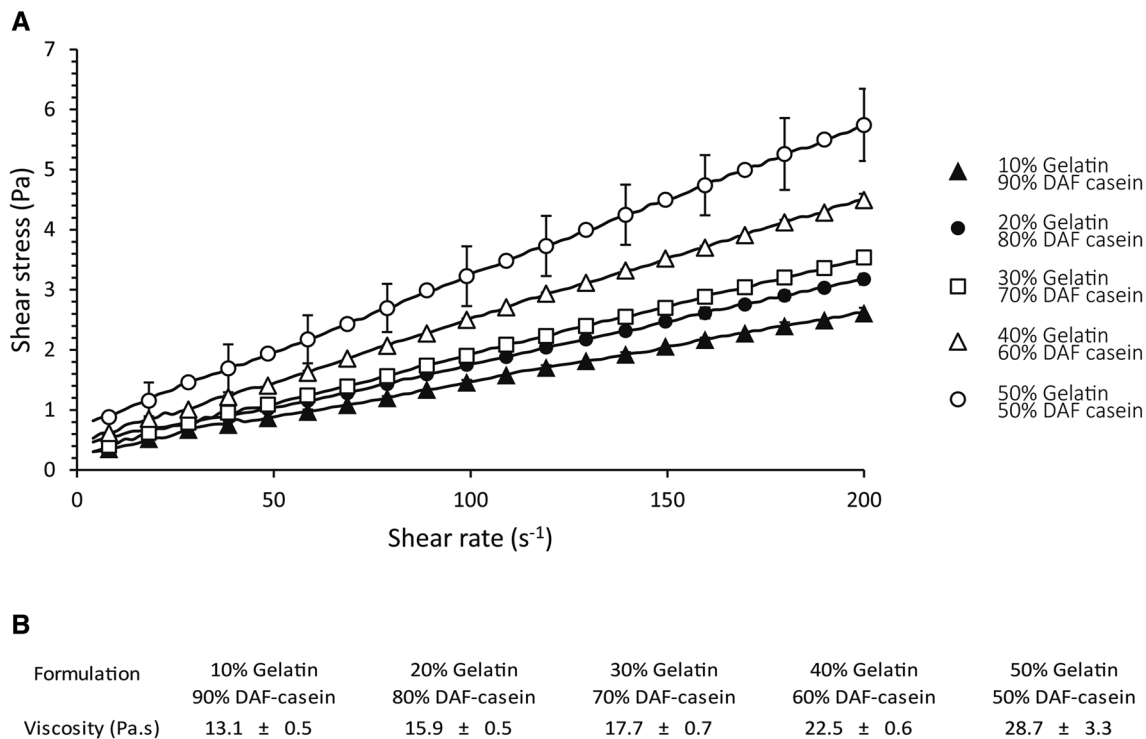
Results and Discussion

Rheological Comparison of Composites

The rheological properties of a DAF-casein solution had previously been compared to that of a solution of reconstituted skim milk powder [15]. This showed that reconstituted DAF-casein possessed a considerably higher viscosity when compared to reconstituted skim milk powder, although both suspensions had a similar fluidic behaviour. It was postulated that this increase in viscosity may be due in part to the incorporation of intermolecular crosslinks, which was supported by polyacrylamide gel electrophoresis and mass spectrometry analysis.

The incorporation of three additional biopolymers, NaCMC, gelatin and κcarrageenan were examined in order to assess their potential effects on the rheology and morphology of DAF-casein solutions and bioplastic films respectively. It has been reported previously that polysaccharide containing bioplastic films exhibit good gas barrier properties and the extended linear structures of most polysaccharides allow for longer interactions between molecules which results in a bioplastic film that is flexible and

robust [17–20]. In addition, gelatin has been shown to have good film forming properties both by itself and in combination with other casein containing materials, in part due to its fibrous structure [12]. The interaction between molecules in a biomaterial is desireable as it typically results in a stronger biomaterial with the molecules interacting together, rather than as separate entities.

Composite solutions of reconstituted NaCMC and DAF-casein were observed to form homogeneous solutions when combined in formulations ranging from 50 to 90% (v/v) DAFcasein with the remainder comprising of NaCMC. All combinations were viscous, and showed a shear thinning profile when the rheological properties were examined (Fig. 1a). This differed from DAF-casein alone, which has been shown to have a linear relationship between shear stress and shear rate, consistent with a Newtonian fluid [15]. As expected, the viscosity of the NaCMC and DAF-casein components decreased as the proportion of NaCMC decreased and DAF-casein increased (Fig. 1b). Under the conditions tested, it was not possible to determine whether the change in viscosity was due to an increase in interactions between the molecules or simply due to the presence of NaCMC. At all ratios tested, the solution remained of a pourable consistency, an important consideration in the handling and

Formulation	10% Gelatin 90% DAF-casein	20% Gelatin 80% DAF-casein	30% Gelatin 70% DAF-casein	40% Gelatin 60% DAF-casein	50% Gelatin 50% DAF-casein
Viscosity (Pa.s)	13.1 ± 0.5	15.9 ± 0.5	17.7 ± 0.7	22.5 ± 0.6	28.7 ± 3.3

Fig. 1 Rheological comparison of reconstituted DAF-casein powder and NaCMC composite solutions. **a** Flow curve of composite solutions. For clarity, error bars are only included in the figure at 20 s^{-1} intervals and represent the mean±standard deviation (n=3). **b** Viscosity 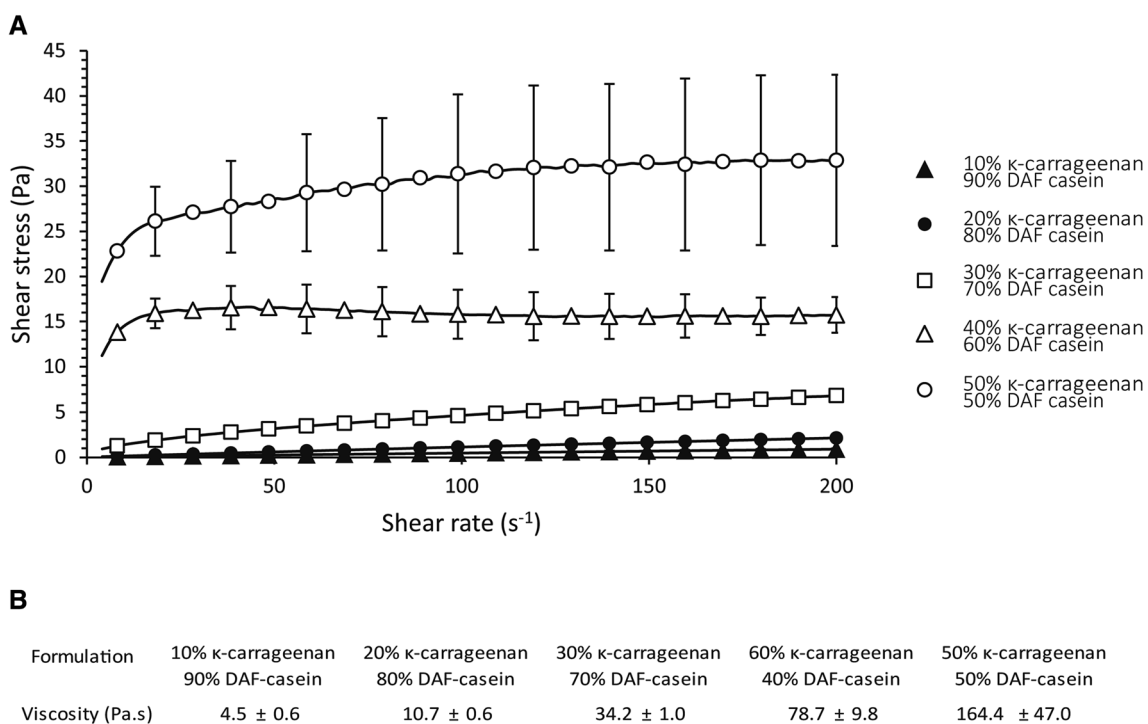of composite solutions at 200 s^{-1}. Errors represent the mean±standard deviation (n=3). All measurements were taken at 20±0.5 °C and after solutions were allowed to stand at room temperature for 12 h. In all cases, the total solids present in samples was 2% (w/v)

processing of the material subsequently for the production of bioplastic films. As the viscosity of composite material solutions increased it was found to lead to the incorporation of air bubbles that were retained in the dried films. While these bubbles could be minimised through sonication of the composite solutions before casting, it proved difficult to completely eliminate them. This may have an effect on the properties of any downstream bioplastic film material produced, such as increased porosity of the film.

Whilst potential applications of the combined use of cellulose derivatives such as NaCMC and whole milk total protein have not been reported, interactions between NaCMC and individual milk proteins have been reported. A study by Cluskey et al. [21] found that NaCMC could form a complex with either α-casein, β-casein or βlactoglobulin through ionic interactions, and that this then resulted in the precipitation of the protein polysaccharide complex. The authors also found that when calcium salts were added to the solution, a higher degree of protein precipitation occurred. However, when EDTA was added no protein precipitation occurred, suggesting that the interaction of milk proteins and NaCMC may be through the mutual interaction of calcium phosphate which stabilises the micellar structure of caseins. When reconstituted DAF-casein powder and reconstituted NaCMC

powder were combined in the present study no precipitation was observed even after the solution was allowed to stand for 24 h. In addition, centrifugation of the sample did not pellet any material.

DAF-casein and κ-carrageenan solution composites were found to form a homogenous blend with no visible separation occurring upon standing. Composite solutions containing 40% or greater κ-carrageenan were observed to form a soft gel when left to stand at room temperature, and as such, the rheological profile of the κ-carrageenan (conducted at 20 °C) was found to change substantially depending on the composition of the composite solution (Fig. 2a). With 40% κ-carrageenan or greater, the composite solution displayed a shear thinning profile, up to the point where the gel structure was lost. From this point, the shear stress remained constant, despite the shear rate continuing to increase under the conditions used. Composite solutions containing 30% or less κ-carrageenan displayed a linear relationship between shear stress and shear rate, similar to reconstituted DAF-casein alone [15]. As composite solutions containing elevated levels of κ-carrageenan formed a gel, they could not be conventionally cast as pourable solutions unless the solutions were first heated. In addition, exposure of the gelling composite solutions to higher shear rates was observed to drastically

Fig. 2 Rheological comparison of reconstituted DAF-casein powder and κ-carrageenan composite solutions. **a** Flow curve of composite solutions. For clarity, error bars are only included in the figure at 20 s^{-1} intervals and represent the mean ± standard deviation (n = 3). **b** Viscosity of composite solutions at 200 s^{-1}. Errors represent the mean ± standard deviation (n = 3). All measurements were taken at 20 ± 0.5 °C and after solutions were allowed to stand at room temperature for 12 h. In all cases, the total solids present in samples was 2% (w/v)

change the behaviour of the material, which is a consideration if the material should be extruded or handled in such a way that significantly increased the shear rate. As with the NaCMC containing composite solutions, the viscosity of the solutions decreased progressively as the proportion of κ-carrageenan present in the composite was lowered (Fig. 2b).
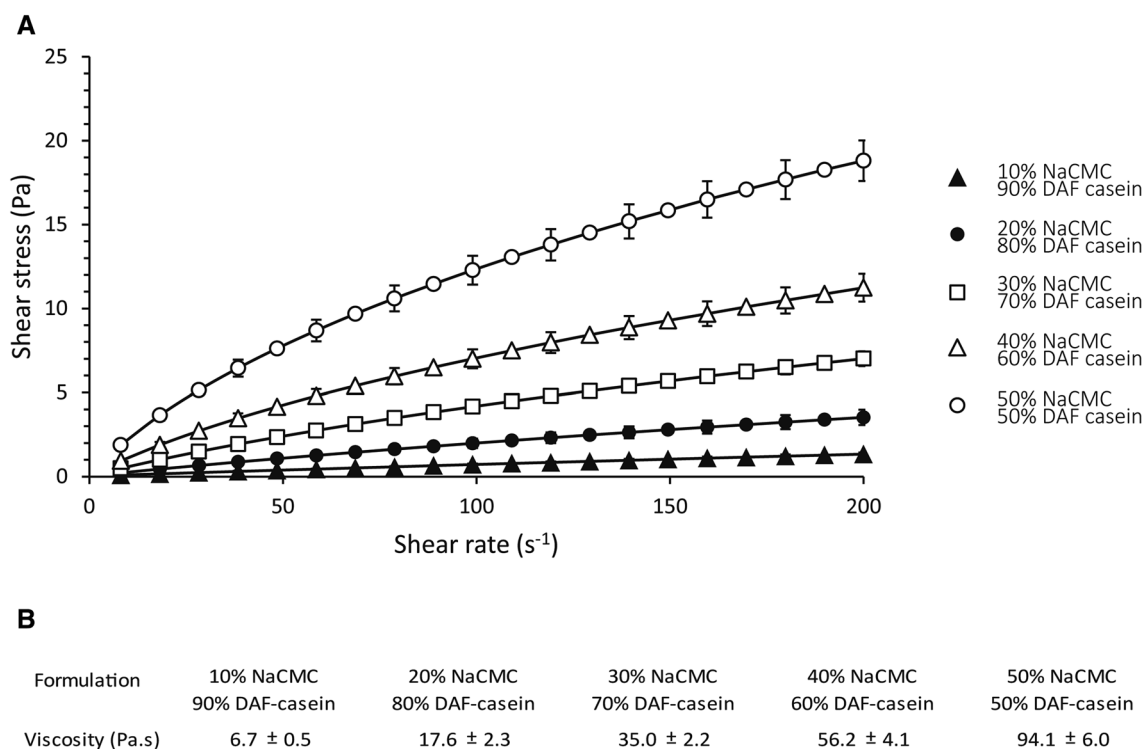
Carrageenan is a commonly available polysaccharide derived from red seaweeds and, like NaCMC, is an anionic polysaccharide with a repeating disaccharide sequence [22]. Gelation of carrageenan occurs when the polysaccharide takes on a helical conformation, which can only occur when the B residue is in the 1-C-4 conformation facilitating gel formation. Stabilisation of the 1-C-4 conformation requires the presence of a 3,6-anhydro bridge to provide steric hindrance. This bridge is not present in all carrageenans, but is a defining feature of both κ- and ι-carrageenans [23].

In addition to gel formation, the helical structure of some carrageenans allows for direct interaction with milk proteins. The helical conformation of κ- and ι-carrageenan results in an increased density of negative charges on the exterior of the helix, which may then form electrostatic interactions with the positive patch between residues 97 and 112 of κ-casein when the pH of the solution is maintained

above the pI of casein [24]. These residues occur within the hydrophilic tail of κ-casein and so are exposed even when the caseins are in their micellar form. The possibility for interactions between caseins and carrageenans have led to the use of carrageenans as a thickener and stabiliser in the production of products such as ice creams, cream cheeses and dairy-based desserts.

DAF-casein and gelatin formed a homogenous composite solution that did not show any form of phase separation. None of the DAF-casein and gelatin composite solutions evaluated were observed to form a gel at room temperature, despite the known gelling capabilities of gelatin. The critical concentration for the formation of a gelatin gel is reported to be between 0.4 and 1% (w/v) total solids depending on the origin of the material [25] and so it is likely that the concentration of gelatin present in the final sample was below the critical gelling concentration.

All of the DAF-casein and gelatin composite solutions that were examined in this study displayed a proportional relationship between the shear stress and shear rate and therefore was the only biopolymer combination tested that maintained the same rheological profile at all gelatin concentrations compared to the DAF-casein alone (Fig. 3). The DAF-casein and gelatin composites also provided a lower

Formulation	10% NaCMC 90% DAF-casein	20% NaCMC 80% DAF-casein	30% NaCMC 70% DAF-casein	40% NaCMC 60% DAF-casein	50% NaCMC 50% DAF-casein
Viscosity (Pa.s)	6.7 ± 0.5	17.6 ± 2.3	35.0 ± 2.2	56.2 ± 4.1	94.1 ± 6.0

Fig. 3 Rheological comparison of reconstituted DAF-casein powder and gelatin composite solutions. **a** Flow curve of composite solutions. For clarity, error bars are only included in the figure at 20 s^{-1} intervals and represent the mean ± standard deviation (n = 3). **b** Viscosity of composite solutions at 200 s^{-1}. Errors represent the mean ± standard deviation (n = 3). All measurements were taken at 20 ± 0.5 °C and after solutions were allowed to stand at room temperature for 12 h. In all cases, the total solids present in samples was 2% (w/v)

viscosity fluid when compared to the composites containing carrageenan and NaCMC, an important consideration for any downstream processing or manipulation of the material such as pouring and extrusion through a fine tube.

Gelatin has been previously combined with casein-containing materials as a thickening agent, and it has been found that, due to opposite electrostatic charges, gelatin may interact directly with κ-casein in the pH range between 6.6 and 8.0 [11, 12]. The pH of a composite solution comprising 50% gelatin and 50% DAF-casein was determined in our study to be 7.5. The inclusion of gelatin in biomaterials has been reported to provide possibilities for enhancing the properties of the resultant composite biomaterial due to the fibrous structure of gelatin allowing for a high degree of flexibility [11, 12, 26]. However, the heterogeneous molecular nature of gelatin leads to some unpredictability in the properties of the material, particularly when comparing gelatin from different sources. One study [27] found that a bioplastic film generated from gelatin prepared from piscine collagen was more soluble when compared to gelatin prepared from either bovine or porcine sources, and that bioplastic films produced from bovine gelatin showed a significantly shorter elongation at break compared to those produced from porcine and piscine gelatin. Bioplastic films formed from gelatin have been previously described as being transparent and to possess a high tensile strength [27]. All gelatins used in the study by Nur Hanani et al. [27] were derived from collagen from the skin of the respective animals. The gelatin used in the present study was derived from porcine skin.

The Use of DAF-Casein for the Production of Bioplastic Films

Reconstituted DAF-casein powder was found to form a cohesive film using a wet casting method under ambient conditions (Fig. 4). This is likely in part due to the self-associating nature of the protein biomacromolecules, whereby casein proteins are known to form micelles due to their amphipathic nature [28]. The method used to produce the films in this study was similar to that used previously to produce thin bioplastic films [29, 30], and was selected due to its simplicity and ability to be scaled up to produce large quantities of bioplastic films. In comparison to reconstituted skim milk powder, the reconstituted DAF-casein powder film had better dimensional and structural integrity as the skim milk powder film cracked while drying. The improved film forming ability of the DAF-casein may be due to the possible higher degree of interaction between the protein components as suggested previously [15].

Whilst it was found that DAF-casein possessed the ability to form a cohesive and homogenous film that had improved characteristics compared to skim milk powder [15], it was noted that the resultant film was brittle and fragile and had

Fig. 4 Comparison of bioplastic films produced from DAF-casein and skim milk powder. DAF-casein bioplastic film produced from 2% (w/v) solution compared to a bioplastic film produced from skim milk powder

limited flexibility. The use of DAF-casein to produce a composite with additional biopolymers was investigated in order to produce a film with improved physical properties. The use of composites including dairy proteins has been explored previously and has been observed to positively influence the mechanical properties of the resultant bioplastic film [11, 31, 32].

Morphology of DAF-Casein Containing Composite Bioplastic Films

SEM analysis of a DAF-casein only bioplastic film showed some irregular material was distributed throughout the surface. This appears to be consistent with previous observations reported in relation to the complex and impure nature of DAF-casein [10, 15]. Additionally, all films that were comprised of 100% of each of the additional biopolymers (NaCMC, κ-carrageenan and gelatin) were found to be brittle and inflexible, however, all were observed to be translucent, in comparison to the opaque appearance of the DAF-casein film.

When low concentrations of reconstituted NaCMC powder were combined with reconstituted DAF-casein powder, some separation between the two components occurred, producing a rough bioplastic film that was also brittle. This occurred despite the solution appearing to be homogenous prior to casting, with no visible precipitation (Fig. 5). This may suggest that some degree of precipitation occurs during the drying process, as reported previously [21]. In addition, the bioplastic film was brittle, like the original bioplastic film containing DAF-casein only. SEM images of this bioplastic film indicated a consistent film with only minor material defects consistent with what was observed in the DAF-casein bioplastic film.

Composite bioplastic films containing 30% or more NaCMC were smooth in appearance and more flexible than bioplastic films of either the DAF-casein or the NaCMC
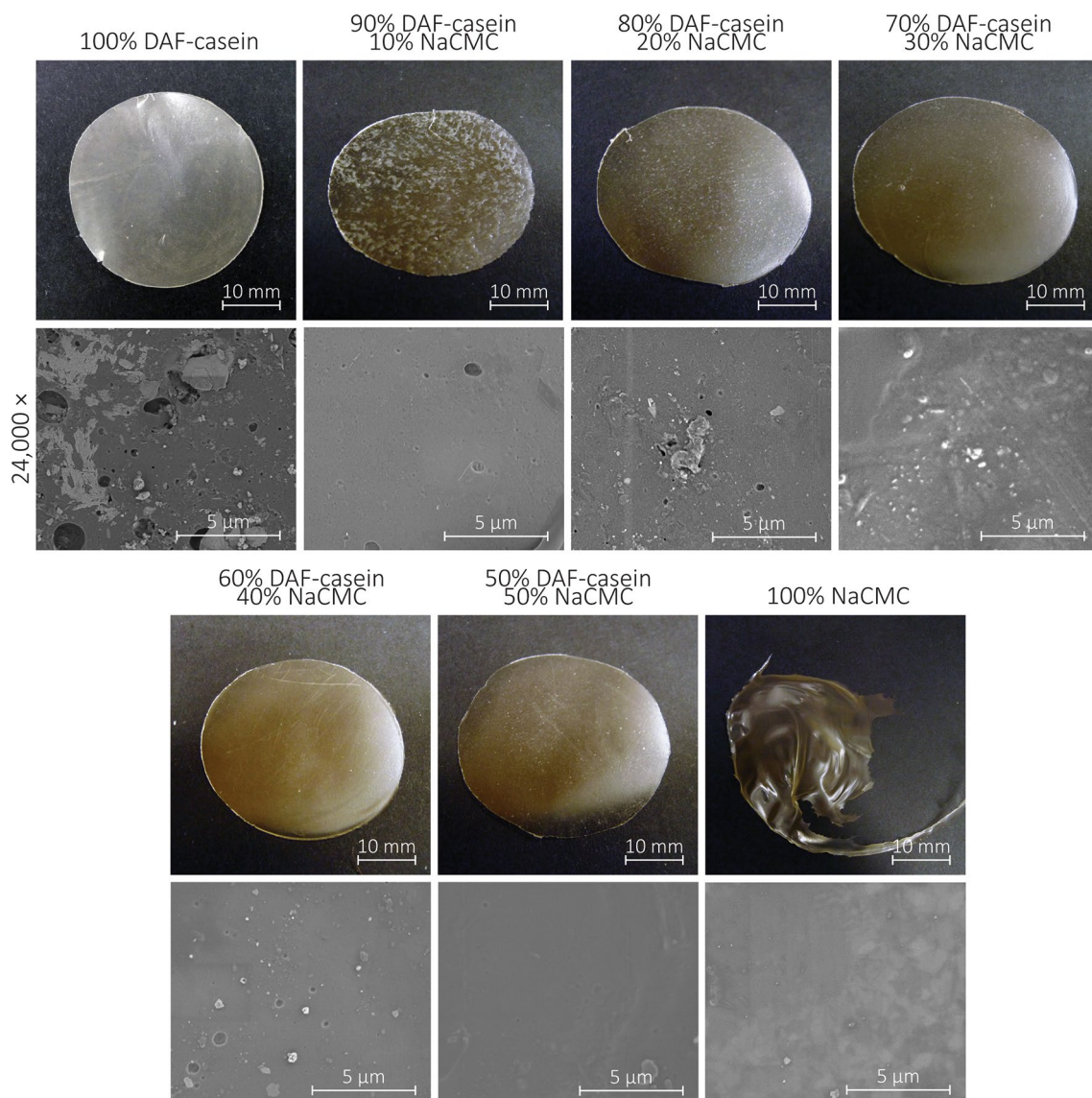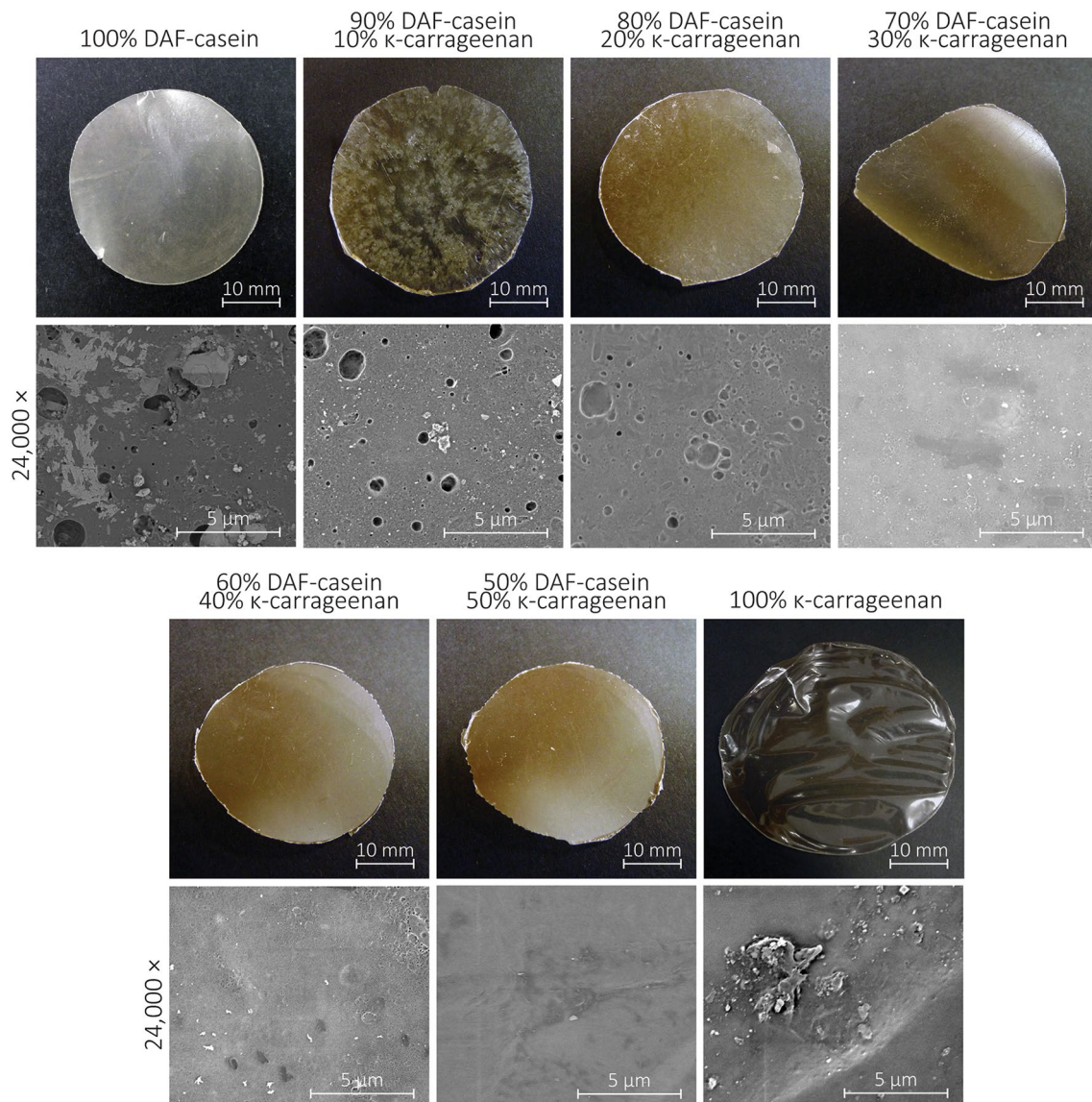
Fig. 5 Morphology of DAF-casein and NaCMC composite bioplastic films with increasing NaCMC concentration. Microscope images generated with SEM. Scale bar indicates 5 μm. All images were collected by applying a voltage 1.0 kV and using a frame time of 9.4 s. All bioplastic films were prepared by blending reconstituted DAF-casein and NaCMC powders at the given ratios to give a final concentration of 2% (w/v) total solids. All bioplastic films were cast to achieve 6.36 mg solids/cm^2

alone. As had been noted previously, the DAF-casein and NaCMC composite solutions showed elevated viscosity and were prone to containing air bubbles (Fig. 2b). In order to reduce the retention of the air bubbles in the solution, all DAF-casein and NaCMC formulations were sonicated for 30 min at 50 °C prior to casting. When cast, all solutions appeared to be free of visible bubbles. Despite this, SEM images showed that some of the bioplastic films appeared to contain small bubbles of less than 1 μm in diameter. This could have a detrimental effect on the physical barrier properties of this bioplastic film. Any observed irregularities in the composites appeared to be consistent with the

appearance of the DAF-casein only bioplastic films rather than as a result of the two components being combined.

When composite solutions containing at least 30% κ-carrageenan with DAF-casein were cast and dried, a cohesive and smooth bioplastic film was formed that was similar in appearance to a 100% DAF-casein bioplastic film, but was more transparent and flexible and could be subjected to tension without appearing to undergo significant deformation (Fig. 6). The inclusion of a higher proportion of κ-carrageenan appeared to increase the strength of the bioplastic film. The apparent increase in strength of the bioplastic film may be due to direct ionic interaction between

Fig. 6 Morphology of DAF-casein and κ-carrageenan composite bioplastic films with increasing κ-carrageenan concentration. Microscope images generated with SEM. Scale bar indicates 5 μm. All images were collected by applying a voltage 1.0 kV and using a frame time of 9.4 s. All bioplastic films were prepared by blending reconstituted DAF-casein and κ-carrageenan powder at the given ratios to give a final concentration of 2% (w/v) total solids. All bioplastic films were cast to achieve 6.36 mg solids /cm²

carrageenans and caseins allowing the material in the bioplastic film to behave as one, rather than existing as separate entities [33]. When a composite bioplastic film was prepared using less than 20% κ-carrageenan some separation of components was apparent and roughness was observed on the surface of the bioplastic films. In turn, the bioplastic films were more brittle and had decreased flexibility as the proportion of κ-carrageenan present was reduced.

When composite bioplastic films containing various ratios of κ-carrageenan and DAF-casein were subjected to SEM imaging they appeared to contain pores, particularly when the lower concentrations of κ-carrageenan were used.

It is unlikely that this was due to air bubbles trapped in the solution as the composite solutions containing low concentrations of κ-carrageenan were much less viscous than those containing higher concentrations of κ-carrageenan and did not form a gel. Bioplastic films containing 30% or greater κ-carrageenan appeared much more consistent, although still contained some irregular material features consistent with what was shown in the 100% DAF-casein bioplastic films (Fig. 6).

There has been some previous evaluation of the combination of gelatin with dairy proteins for the production of bioplastic films. Chambi and Grosso [11] explored the use

of gelatin in a composite with caseins in order to produce edible bioplastic films for food packaging. The casein used in the study by Chambi and Grosso [11] utilised technical grade casein, and so some differences would be expected when using a more complex material such as DAF-casein. DAF-casein and gelatin composite bioplastic films retained the discolouration and opaqueness that was observed with a DAF-casein only bioplastic film, however, the flexibility of the film increased. When DAF-casein and gelatin were present in equal proportions, the bioplastic film appeared to become more translucent and glossy (Fig. 7). All of the composite bioplastic films with different proportions of

DAF-casein and gelatin appeared to be homogenous, with the exception of the bioplastic film containing 90% DAF-casein and 10% gelatin. This bioplastic film displayed some haziness on the surface that was not evident in the others, although it did not share the same roughness that was present with bioplastic films containing 90% DAF-casein and 10% NaCMC (Fig. 7).

SEM analysis of the surface morphology of the DAF-casein and gelatin composite bioplastic films revealed that the bioplastic films had very few prominent features, although it was observed that these composite solutions were prone to contain air bubbles, and the apparent size of these
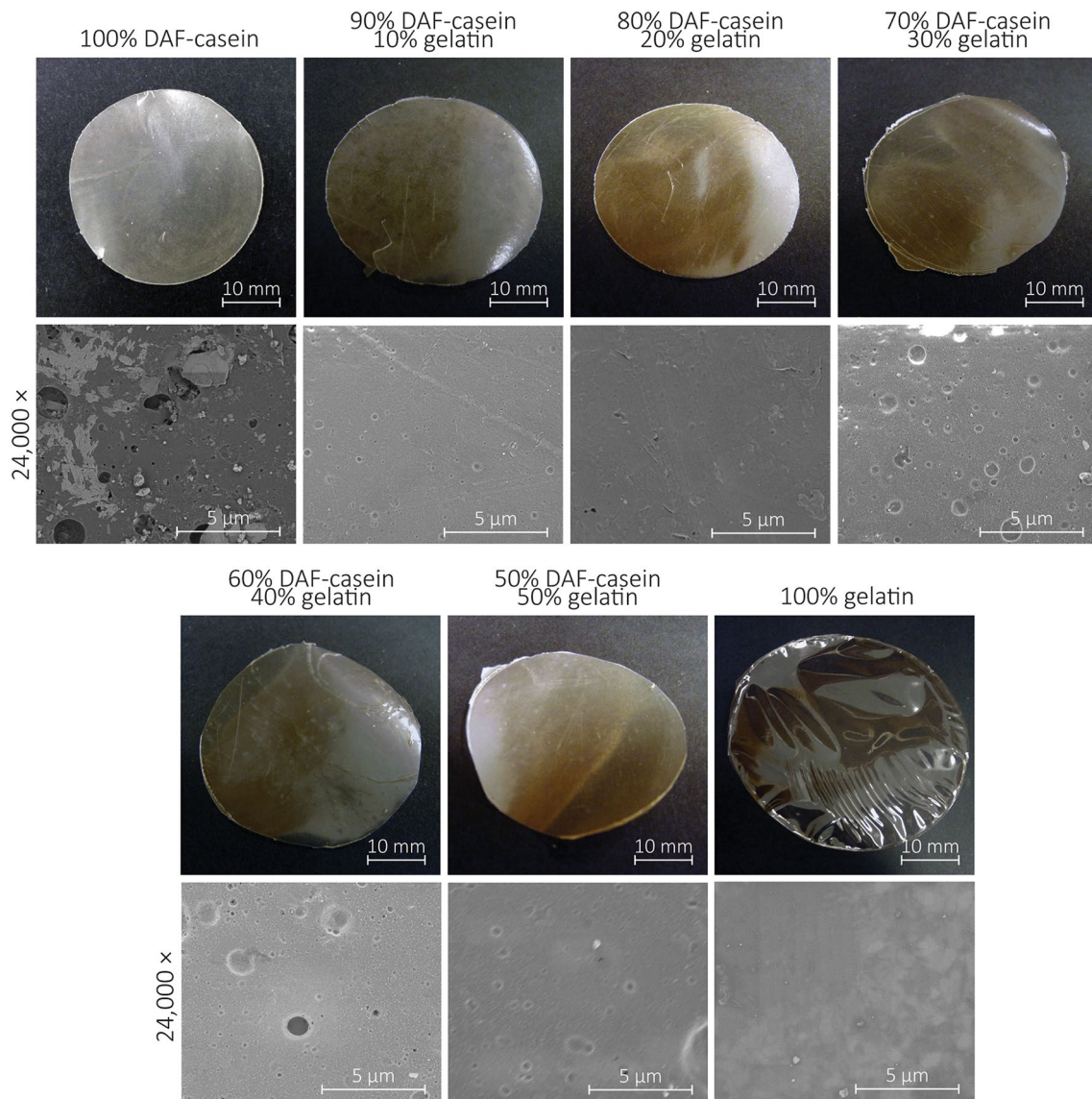
Fig. 7 Morphology of DAF-casein and gelatin composite bioplastic films with increasing gelatin concentration. Microscope images generated with SEM. Scale bar indicates 5 μm. All images were collected by applying a voltage 1.0 kV and using a frame time of 9.4 s. All bioplastic films were prepared by blending reconstituted DAF-casein and gelatin powder at the given ratios to give a final concentration of 2% (w/v) total solids. All bioplastic films were cast to achieve 6.36 mg solids/cm^2

bubbles in the dried bioplastic films appeared to increase as the proportion of gelatin increased (Fig. 7). Although this may be advantageous for an application where a breathable packaging is sought, it would not be suitable for an airtight packaging application, such as where a product is prone to spoilage by oxidation. Unlike the DAF-casein and NaCMC composite bioplastic films, the DAF-casein and gelatin composite bioplastic films did not exhibit any of the irregular material features that were apparent in the DAF-casein only bioplastic films.

Gelatin was the only non-polysaccharide derived biopolymer that was evaluated for its ability to form a composite with DAF-casein. A limitation of polysaccharides is that they are constructed of a repetitive sequence of sugar molecules with very few novel functional groups. This reduces the possibilities for non-covalent interactions with other biopolymers in a composite bioplastic film. A bioplastic film is usually more robust when components directly interact with one another through either hydrogen, ionic or covalent bonds to form a molecular network rather than existing as separate entities. Protein based biopolymers, however, contain a wide variety of functional groups that may increase the possibility of forming additional intermolecular crosslinks [34].

Water Stability of DAF-Casein Containing Composite Films

The stability of a bioplastic film in an aqueous environment is key to directing the potential application of a material. To compare the response to an aqueous environment, DAF-casein and biopolymer composite bioplastic films were submerged in Milli-Q water at room temperature and any dimensional changes were noted. All films analysed were 60% DAF-casein with the remainder 40% comprised of the additional biopolymer. This ratio was selected as it provided a smooth bioplastic film with no apparent inconsistencies across all of the biopolymers investigated.

The DAF-casein only bioplastic film was observed to have poor physical and dimensional stability when exposed to water. Initially the 100% DAF-casein bioplastic film appeared to remain intact, although it had rolled in at the edges (Fig. 8). However, by 1 h immersion in water, the bioplastic film had swelled noticeably to occupy the majority of the petri dish, and could not be handled without disintegrating. The instability of protein based bioplastic films containing casein, whey and soy protein has been reported previously [35–37]. Protein based bioplastic films typically form amorphous three dimensional arrays, which lack any intermolecular covalent bonds for stabilisation, are not water repellent, and hence result in a high degree of water uptake that destabilises the structure [35].

When immersed in Milli-Q water, the DAF-casein and NaCMC composite bioplastic film was found to swell considerably (Fig. 8). In the first 30 min the bioplastic film rippled and became unstable to the point where it could not be handled without disintegrating. After 1 h the bioplastic film had completely dissolved. It is known that all salts of CMC are readily miscible in water due to the presence of both hydroxyl and carboxyl groups, and the inclusion of CMC in a soy protein isolate bioplastic film has previously been shown to increase the solubility of the bioplastic film compared to a film of the protein alone [2].

Similar to DAF-casein and NaCMC composite bioplastic films, DAF-casein and gelatin composite bioplastic films were observed to expand after 30 min of being immersed in Milli-Q water at room temperature. However, while the NaCMC-containing bioplastic film rippled during this swelling phase, the gelatin containing bioplastic film swelled in a more radial manner. After 1 h, the gelatin film had swelled further but remained intact, however, it no longer had structural integrity and could not be manipulated without breaking. It has previously been shown that the inclusion of gelatin into a starch based bioplastic film resulted in an increase in the water solubility of the bioplastic film [26]. The authors proposed that this is due to the hygroscopic and water holding capacity of gelatin. Although in the present study the DAF-casein and gelatin composite bioplastic film did not appear to solubilise in Milli-Q water any faster than bioplastic films containing only DAF-casein, it did show the largest dimensional expansion out of all of the bioplastic films tested.

Of the composite bioplastic films, the κ-carrageenan containing composite bioplastic film remained stable for the longest time when immersed in Milli-Q water. After 30 min the composite bioplastic film containing κ-carrageenan did not appear to have changed dimensionally, and could still be handled and removed from the water with no apparent dimensional instability and structural damage. However, after 1 h in Milli-Q water, much of the bioplastic film had dissolved with only a small amount of the original bioplastic film remaining and this could not be handled. The higher perceived stability of the DAF-casein and κ-carrageenan bioplastic film was perhaps due to the direct interaction and physical cross-linking that can occur between casein and κ-carrageenan imparting a higher degree of stability [24]. However, despite this decreased solubility, the bioplastic film still dissolved over a reasonably short time duration and would not be suitable for use as a long term packaging material under humid or damp conditions.

Due to the rapid structural changes observed with all composite bioplastic films, no attempts could be made at quantifying the uptake in water. The water solubility of each of the composite films would make them unsuitable for any application which requires the exposure to water or water
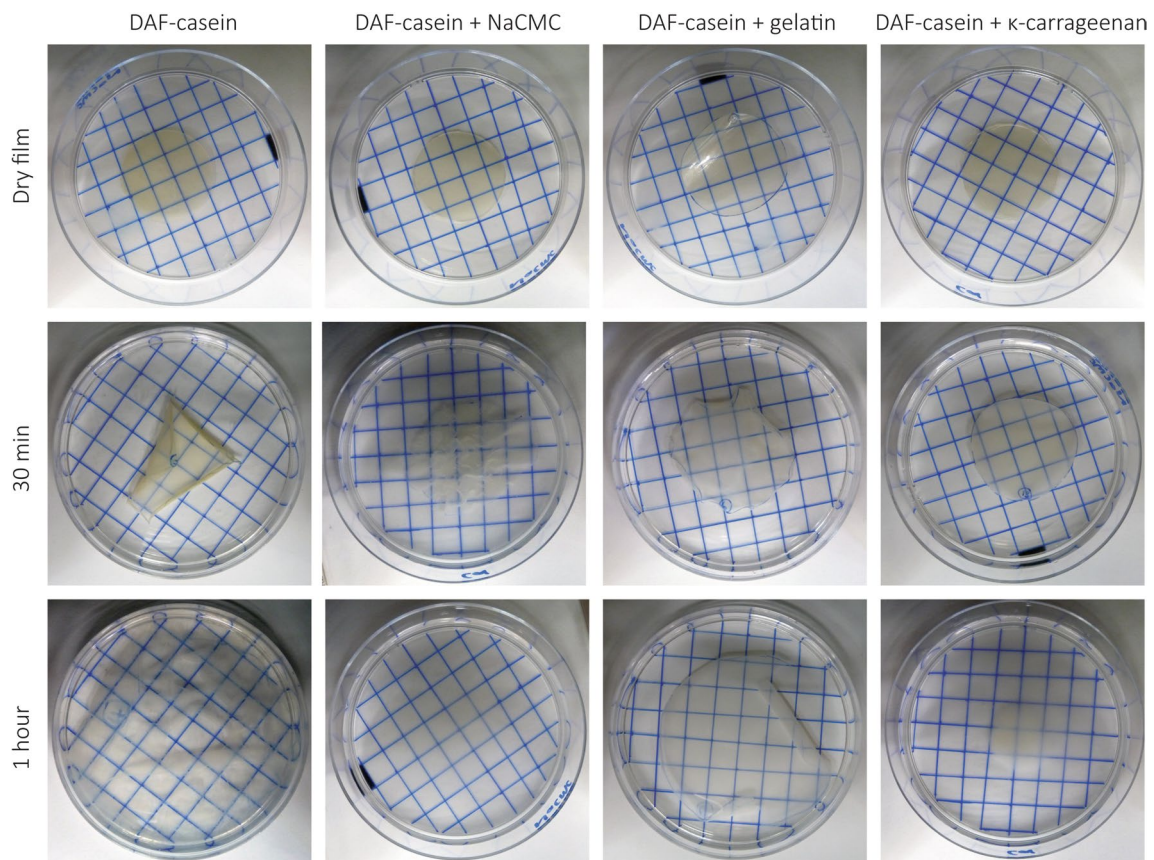
Fig. 8 Water swelling of 60% DAF-casein and 40% biopolymer composite bioplastic films. Films (35 mm diameter) were submerged in 15 mL Milli-Q water at ambient temperature for up to 1 h. Grids represent approximately 1 cm². **a** DAF-casein; **b** DAF-casein and NaCMC; **c** DAF-casein and gelatin; **d** DAF-casein and κ-carrageenan. All bioplastic films were prepared by blending reconstituted DAF-casein and an additional biopolymer to give a final concentration of 2% (w/v) total solids. All bioplastic films were cast to achieve 6.36 mg solids/cm2. All composite films are comprised of the same DAF-casein to biopolymer ratio

vapour. The use of crosslinkers to form both covalent and non-covalent bonds has been found to stabilise the structures of bioplastic films by forming additional intermolecular interactions, and hence decrease water solubility [29, 38]. Therefore, the next stage will be to study the effect of crosslinkers and plasticisers on the properties of the DAF-casein containing composite bioplastic films.

Conclusions

An underutilised high volume casein-rich dairy co-product, referred to as DAF-casein, which can be harvested from dairy factory wastewater, presents an opportunity to evaluate the production of biomaterials, including bioplastic films. As reconstituted DAF-casein powder alone was found to produce a brittle film, the incorporation of additional biopolymers was evaluated for the ability to enhance the properties of the bioplastic films. Rheological studies showed that each of the biopolymers that were combined

with DAF-casein modified the rheological properties of the composite solutions in different ways, and most of the composite solution combinations remained pourable at room temperature. The morphology of the resultant composite bioplastic films did not change dramatically, however, some bioplastic films showed the inclusion of small air bubbles which may affect the permeability and barrier properties of the films, which could be a consideration for some packaging applications. Interestingly, the water solubility of the composite bioplastic films was improved compared to that of the DAF-casein only bioplastic film, however, none of the bioplastic films produced had the ability to withstand changes to their structural integrity when placed in Milli-Q water. This study has shown the potential of DAF-casein in biopolymer composites for the production of biomaterials including bioplastic films. The next stage will be to evaluate the ability of crosslinkers and plasticisers to enhance the physical properties of the DAF-casein and biopolymer composites, with the aim to make them more suitable for use in various biomaterial applications.

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