

Swelling Behavior and Mechanical Stability of Composite Fibers Prepared from Casein Micelles and Calcium Alginate

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Milk casein micelles are natural nanocarriers for poorly soluble calcium phosphate and can be used to stabilize and functionalize composite structures due to their excellent gelling properties. This study shows that alginate and native casein micelles can be processed into stable composite fibers by extrusion in a calcium-rich coagulation bath. The influence of micellar casein content on the swelling of fibers in decalcifying media is analyzed and the effects of the treatment on the mechanical properties are investigated. Pure alginate fibers swell strongly in trisodium citrate solutions due to calcium removal, whereas alginate-casein interactions in composite fibers significantly reduce this strong swelling. In acidic solutions such as citric acid and glucono- δ -lactone, pure alginate fibers become softer and lose strength. If the amount of micellar casein in the fiber is increased, it can be observed that the swelling capacity decreases during the acid treatment, but the extensibility increases significantly in mechanical tests after the treatment. However, citric acid also stabilizes the fiber structure through hydrogen bonding and makes the fibers more flexible, which leads to a greater reduction in swelling and allows for higher breaking strains, compared to glucono- δ -lactone.

1. Introduction

Caseins as the main protein component of milk offer great potential for the production of novel, sustainable, biocompatible, and degradable functional composite materials due to their gel-forming properties.^[1,2] Cow's milk contains α_{S1} -, α_{S2} -, β -, κ -caseins, which can be considered as intrinsically disordered proteins.^[3] Under physiological conditions, caseins exist together with colloidal calcium phosphate as spherical association colloids. These so-called casein micelles have diameters between 50 and 300 nm and are essentially stabilized internally by two interactions.^[4,5] On the one hand, stabilization occurs through

hydrophobic interactions between casein molecules.^[6] On the other hand, calcium-mediated contacts exist between clusters of phosphoserine residues in the casein primary structure and colloidal calcium phosphate,^[7] which can be reduced by lowering the calcium content via chelators or by lowering the pH.^[8] In addition, an outer κ -casein surface layer also contributes to the colloidal stability of the micelles, which shrinks and eventually collapses when the pH is lowered.^[9] Under neutral conditions, it sterically stabilizes the casein micelle, protecting it from aggregation and gel formation.^[10]

So far, it has not been possible to directly spin native casein micelles. However, dissociated casein can be converted into regenerated protein fibers using alkaline solutions. In the past, environmentally harmful substances such as formaldehyde and aluminium compounds were used to cross-link the fibers, whereas today an environmentally friendly method using citric acid, CA is used.^[11] However, fibers from micellar casein (MC) can be produced by cutting off a portion of the κ -casein surface layer by enzymatic renneting of casein micelles in the cold, which potentially allows them to aggregate without losing their colloidal stability under these temperature conditions. Aggregation occurs only after extrusion into a warm, calcium-rich coagulation bath, which produces edible micellar rennet casein fibers without the use of further chemicals.^[12] These fibers have a characteristic network structure stabilized by calcium-phosphate contacts, with higher calcium concentrations resulting in a denser structure that swells more slowly.^[13] However, a disadvantage is that the fibers swell strongly and become unstable after the addition of organic acids, including the aforementioned cross-linking agent CA.^[14,15] In addition, the production process is complex due to the previous enzymatic structural modification of the casein micelles at low temperatures. To enhance the mechanical properties of casein-based materials, combining them with a second polymer network has proven effective, as demonstrated in double-network hydrogels.^[16] Recent studies have also highlighted the functional potential of calcium-stabilized polymer systems and casein-based materials in various applications. Casein-alginate hybrid gel filaments with a bundled structure, formed via calcium crosslinking under laminar flow, have been proposed for applications in biomimetic chemistry, as biodegradable scaffolds for cell engineering, and as carriers for the release of phys-

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biologically active substances.^[17] Additionally, recent research on protein/calcium alginate composite gels has demonstrated the potential of calcium-crosslinked systems in meat analogs, where their structural stability makes them promising candidates for mimicking the texture and behavior of real meat.^[18] Recent advancements in 3D food printing using sodium caseinate and sodium alginate blends have enabled the creation of fibrous microstructures, offering new opportunities for the development of meat analogues.^[19] A recent review highlights that casein-based gels combine biocompatibility, mechanical strength, and functional versatility, making them promising for applications in food, biomedical, and sensing fields.^[20] Taken together, these findings highlight the importance of calcium-mediated interactions and protein-polymer architectures in the development of biofunctional materials.

A promising approach for producing stable and sustainable fibers is the use of polysaccharides in combination with casein. Calcium can form cross-links with phosphoserine residues and stabilize the fiber structure through additional chain entanglements, as demonstrated with caseinate fibers.^[21] Alginate is particularly interesting because it forms strong ionic interactions with calcium, which strengthens the fiber structure and makes it a sustainable fiber-forming material with a wide range of applications.^[22–24] Alginate can be obtained from sustainable raw material sources and is characterized by excellent biocompatibility and degradability, low toxicity, and favorable hygroscopic properties. It consists of blocks of (1,4)-glycosidically linked β -D-mannuronate and α -L-guluronate, with the blocks consisting of consecutive G and M residues or alternating between the two.^[25] Alginate gels by cross-linking with calcium ions, forming a characteristic egg box structure.^[26]

Fibers made of alginate and caseinate could be produced by Ca^{2+} chelation under laminar flow conditions in a capillary. Casein adsorbed into the coiled alginate chains, weakening the diaxial hydrogen bonds and causing the alginate material to become more flexible.^[17]

Alginate-casein gels were prepared by adding glucono- δ -lactone (GDL) with varying alginate concentrations. The gel hardness initially increased with increasing alginate content and then decreased at higher concentrations, which was attributed to structural transitions and interactions between casein and alginate at pH values below the isoelectric point of the caseins. At low alginate concentrations, the casein network dominated, whereas at higher alginate concentrations, a bicontinuous structure was formed and the gelation time was shortened.^[27]

In a further study, interpenetrated double gels were prepared by an acid-induced sequential gelation process in which the alginate gel was formed first by the release of calcium ions from calcium citrate, followed by the formation of the casein gel at pH values below 4.8. These double gels exhibited higher hardness and better water holding capacity than simple composite gels.^[28]

Gel formation and stability of calcium-based gels can be influenced by the use of chelating and decalcifying agents. It was found that the addition of citrate did not alter the properties of alginate gels, whereas the addition of CA significantly reduced the tensile strength and elastic modulus.^[29] In contrast, casein micelles are demineralized by the addition of citrate, which indirectly dissolves colloidal calcium phosphate, causing, for ex-

ample, casein micro-particles to swell more and disintegrate faster.^[30,31]

In this study, for the first time, we produce composite fibers from a mixture of MC and alginates by extrusion into a calcium-rich coagulation bath. This process ensures that the alginates are linked together by ionotropic gelation via the strong egg box motif. We are testing how we can modify the polymer interactions by varying the casein content and post-treatment in decalcifying media to specifically alter the mechanical properties of the fibers. Potential future applications of the fibers could include their use in meat analogs, controlled release systems and wound dressings, particularly where responsiveness to environmental factors such as pH and decalcification is desirable.

2. Results and Discussion

2.1. Microscopic Study

Pure calcium alginate and MC alginate composite fibers were prepared by a simple spinning method^[32] using a 3% (w/w) sodium alginate solution prepared in deionized water, with or without added casein micelles, extruded via a capillary into a calcium-rich coagulation bath. **Figure 1** shows confocal laser scanning microscopy images of the MC-alginate composite fibers compared to the pure alginate fiber, all in the dry state. It can be seen that the caseins are stained cyan blue by the dye 8-hydroxyphenyl-1,3,6-trisulfonic acid (HPTS), which can be explained by the binding of the deprotonated dye to the positively charged amino acids Arg and Lys.^[33] In contrast, the pure alginate fiber, which is consistently negatively charged at neutral pH, is not stained, as shown in the control sample in **Figure 1a**.

It can be clearly seen that the fiber is made up of individual strands, which are made visible by the spherical caseins aggregates.

Such particles are also observed in casein gels formed by calcium, acid, or enzymatic destabilization.^[34] Based on previous studies, it is plausible that alginate forms the strands to which casein aggregates then bind, with the density of the protein on the surface increasing with increasing MC content. In SEM images, these alginate strands are still visible at lower MC concentrations, but become increasingly less distinct as the MC content increases (see **Figure S1**, Supporting Information). These results suggest that MC may intercalate between alginate strands, thereby reducing the visibility of the fiber morphology observed in SEM images. There is no emission from the pure alginate fiber itself (**Figure 1a**). On the one hand, it can be observed that the fiber strand is still interrupted by individual uncolored spots at 3% MC (**Figure 1c**), which is less pronounced at 4% MC (**Figure 1d**). On the other hand, we observe lighter appearing continuous patches, i.e., casein-rich islands, which become more frequent with increasing casein concentration on the fiber surface.

2.2. Analysis of the Swelling Behavior

For the swelling tests, fibers were prepared from solutions containing 3% (w/w) sodium alginate, originally dissolved in deion-

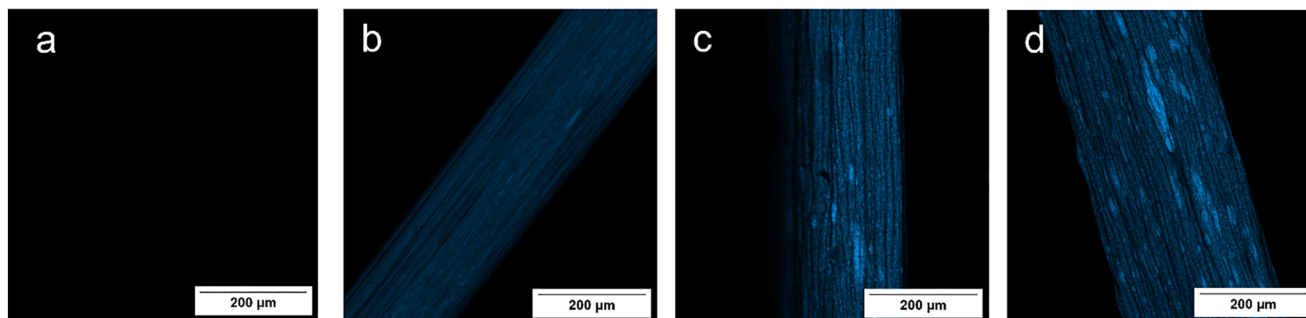


Figure 1. Confocal laser scanning microscope images of dry MC-alginate composite fibers with different MC contents, 0% a), 1% b), 3% c), and 4% d).

ized water, and 0.5%, 1%, 2%, 3%, or 4% (w/w) casein micelles, originally dissolved in SMUF buffer. After preparation and drying, these fibers were immersed in four different solutions – 1 M Trisodium citrate, TSC (pH 8), 1 M CA (pH 2.3), 1 M GDL (pH 2) and water (pH 7) – and the resulting size change was analyzed as a function of time. The increase in fiber width due to swelling and model fits are shown in **Figure 2a–c** for the pure alginate fiber and the composite fibers with 1 and 3% MC. The swelling data for all fiber preparations in all solutions, together with the model fits, can be found in **Figure S2** (Supporting Information).

For all the fibers produced, only the swelling curves in water showed a single-phase profile (**Figure S2d**, Supporting Information), while a two-phase swelling behavior was observed in the other solutions tested. This is further illustrated in **Figure S3** (Supporting Information), where the swelling curves linearized according to the Schott model show a single-phase profile only in water (d), while the other media (a–c) show clear deviations from linearity. These results suggest that the first phase of swelling is due to fiber hydration, while the second phase is probably caused by an additional destabilizing mechanism that does not occur in pure water. Swelling was always most pronounced in TSC and least pronounced in water. In the acidic media CA and GDL, the final swelling values after 600 s were always slightly higher than in water, but only slightly higher overall. **Figure 2** shows that the swelling in TSC is approximately halved with increasing MC con-

tent. Only the 3% MC fiber remained visible throughout the observation period, while the change in size of the 1% MC fiber was no longer detectable under the microscope after 400 s due to lack of contrast. With pure alginate, the fiber was no longer visible after 200 s due to strong swelling. In fact, the fibers could not be harvested after the TSC treatment due to the weak structural cohesion and proved unsuitable for further mechanical tests.

To further investigate the influence of casein content on the increase in fiber width in the different solvents, all swelling curves were analyzed using a rate model. The swelling curves in water were described by the Schott model (Equation 2), while the biphasic swelling curves in the decalcifying media were analyzed by an extended Schott model (Equation 3). The extended model is defined by six independent parameters. The rate constants k_1 and k_2 describe the swelling rates of the two phases, while the plateau values $S_{1,\infty}$ and $S_{2,\infty}$ indicate the maximum fibre width in percent reached at the end of the respective swelling processes. Two further parameters, t' and k_t , describe the transition between the two swelling processes via an exponential function (Equation 5). This approach is based on the hypothesis that dry casein fibres are first hydrated in acidic solutions. Subsequently, a second swelling process gradually occurs via the transition function, which is due to the weakening of calcium phosphate bridges.^[13]

When looking at the parameter values in **Figure 3**, a large variability of the fit values for pure alginate fibers and composite

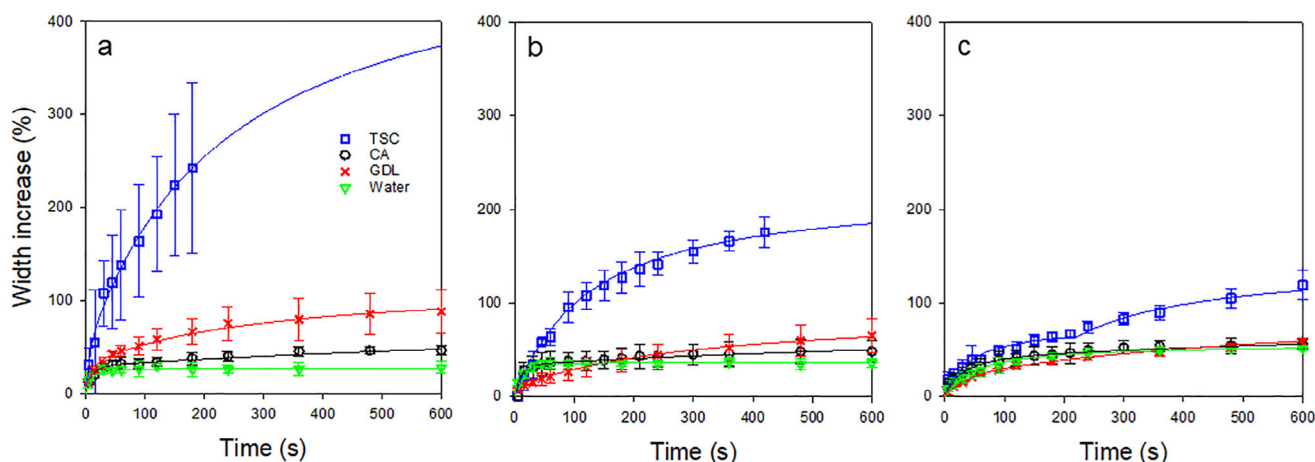


Figure 2. Swelling curves of fibers with 3% alginate with 0% MC a), with 1% MC b), and with 3% MC c) in different solutions. The mean values of three measurements and the corresponding model fits are shown.

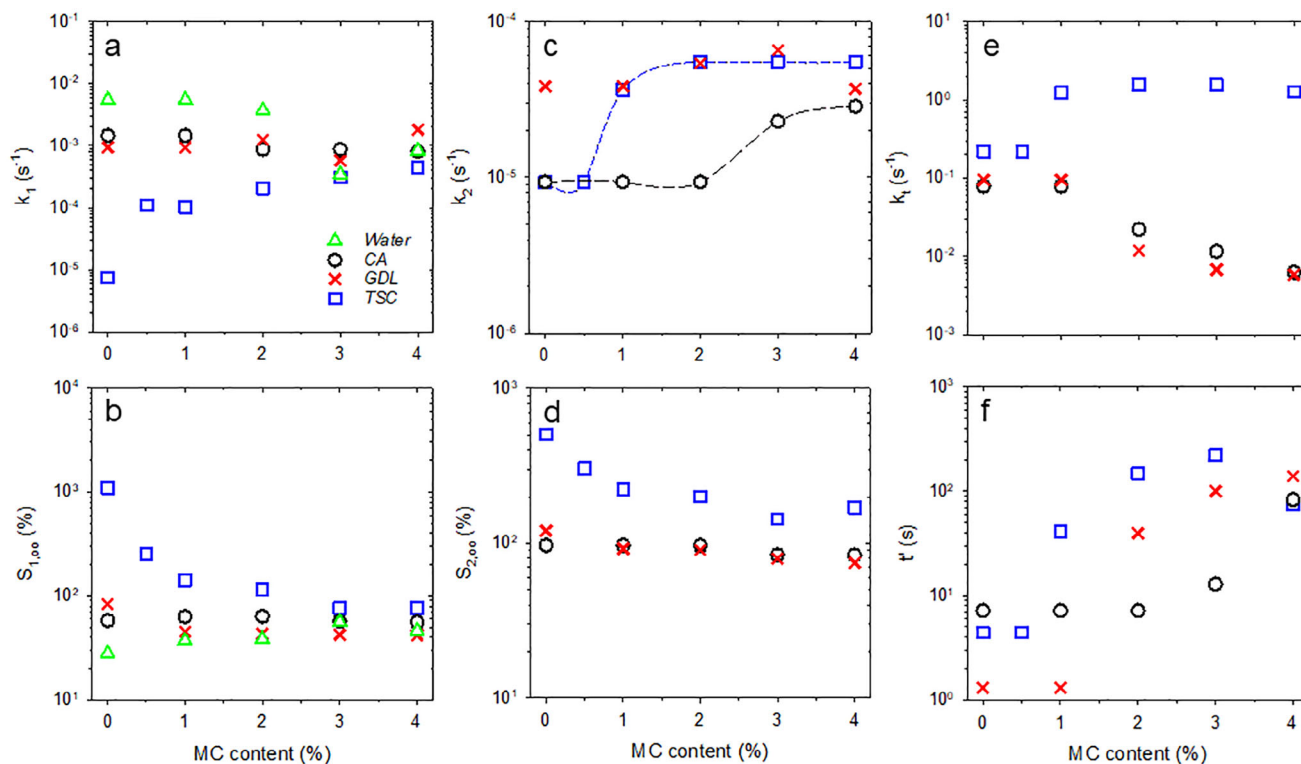


Figure 3. Values of the fit parameters from the rate models (Equations 2 and 3) used to analyze the swelling curves of alginate/MC fibers are shown as a function of MC content in four different swelling media: water, CA, GDL, and TSC. a) rate constant of the first swelling process; b) plateau value of the first swelling process; c) rate constant of the second swelling process; d) plateau value of the second swelling process; e) rate constant of the transition function; f) delay time of the transition function.

fibers with a low MC content is noticeable. With an MC content of 4%, however, the values are close together, with the exception of the transition rate k_t for TSC. From this, it can be concluded that the network structure of the fiber determines the swelling at high MC content due to strong alginate-casein interactions, whereas with pure alginate or low casein content the swelling is significantly influenced by the type of solution and the pH value.

In water, where only a swelling process (hydration of the fiber) takes place, the rate decreases from $k_1 = 0.006$ to 0.0008 s^{-1} as the casein content increases from 0% to 4% (Figure 3a; Table S1, Supporting Information). This observation can be explained by a denser surface structure resulting from the increased accumulation of casein on the surface of the alginate fibrils (Figure 1; Figure S1, Supporting Information). At the same time, casein supports the hydration of the fiber through a large number of hydrophilic groups, which is noticeable in an increase in $S_{1,\infty}$ with increasing casein content (Figure 3b).

In GDL and CA solutions, the swelling rate at low MC content is lower than in water, which can be explained by the lower proportion of deprotonated carboxyl groups on the alginate and a higher ionic strength in the acidic solutions. With increasing casein content, the swelling rate decreases only slightly, while the first swelling level $S_{1,\infty}$ remains largely constant.

In TSC, the initial swelling process of the pure alginate fiber is very slow with a rate $k_1 = 7.503 \cdot 10^{-6} \text{ s}^{-1}$. The low value could be due to the significantly higher ionic strength resulting from

3 dissociating sodium ions of TSC in water. At the same time, $S_{1,\infty}$ increases by a factor of 1000, indicating that the strong ionic cohesion between the calcium alginate strands is already significantly weakened during the first swelling process. Removal of calcium from the network by complex formation with citrate eventually leads to complete dissolution of the alginate network,^[35] so that no alginate fibers could be harvested after treatment in our experiments. The swelling rate increases with increasing MC content in the fiber, which can be explained by the large number of hydrophilic groups in casein. At the same time, $S_{1,\infty}$ decreases, which can be attributed to a network structure stabilized by casein. In TSC, the calcium chelating activity results in the irreversible removal of micellar calcium phosphate, causing the casein micelles to disintegrate,^[36] and less citrate is available to destabilize the alginate. The dissociated casein can adsorb onto the alginate chains, stabilizing the network structure and making it more flexible.^[17]

Equation 3 predicts similar final $S_{2,\infty}$ values of around 100% for both GDL and CA for all fiber preparations, with a slight trend towards lower values with increasing MC content. In general, it can be assumed that the fiber structure is stabilized by casein during acidification as the casein micelles gel during the pH drop^[37] and attractive interactions between alginate and casein arise. The latter occurs when the pH falls below the isoelectric point of casein ($\text{pH} \approx 4.6$),^[27] which can be assumed to occur during the second phase of swelling in CA and GDL. This is also clearly shown

by the swelling curves in GDL, whose values decrease with increasing casein content within the observation period of 600 s (Figure S2c, Supporting Information).

For CA, a slightly greater swelling of the fiber is observed at an MC content of 4% compared to GDL (Figure S2b, Supporting Information). After 600 s, a swelling value of about 55% is reached compared to about 40% in GDL. However, in GDL more amino acid residues in the casein are protonated at pH 2 and stronger casein-alginate interactions are possible than in CA at pH 2.3, which explains the lower swelling values.

However, Figure S2b (Supporting Information) also shows that the swelling values in CA decrease as the MC content in the fiber decreases. The rate k_2 for fibers with MC content up to 2% is reduced to a quarter of the rate in GDL (Figure 3c), which is a consequence of the additional stabilization. This effect could be due to the strong hydrogen bonds that CA forms with sodium alginate. This improves the interaction between the alginate chains and reduces the water sensitivity of alginate-based films.^[38] In contrast, when the MC content in the fiber is increased to 3% or more, the rate increases significantly and reaches almost the same value as the swelling in GDL at 4% MC content (Figure 3c, black dashed line). This could indicate that MC at higher concentrations prevents the hydrogen bonds with CA and thus weakens the interaction between the alginate chains.

Differences between the swelling in CA and GDL are also evident in the values of t' , which defines the transition time between the swelling processes (Figure 3f). For CA, t' is significantly delayed for fibers without and with 1% MC compared to GDL, which is due to the stabilizing effect caused by the formation of hydrogen bonds. However, from a MC content of 2%, the lag time in GDL becomes longer, which can be explained by the absence of the chelating effect of GDL on calcium ions that exists in CA. As a result, the calcium in the casein micelles is released more slowly in GDL, which stabilizes the fiber structure for longer.

In contrast, the rate of transition k_t in CA and GDL is similar for all fiber preparations (Figure 3e). It decreases with increasing MC content, which is due to the increasing complexity of the fiber structure with increasing casein content, making the transition wider.

In TSC, the pure alginate fiber expands to a theoretical value of more than 500% of the initial value, decreasing to 200% with increasing MC content. As described above, the structural cohesion of the fibers after TSC treatment was very weak and fiber swelling was not detectable for some preparations over the entire observation period due to lack of contrast.

The strong swelling at low MC levels in the fiber is mainly due to the removal of calcium from the eggbox structures of the alginate, which already occurs in first swelling process in the presence of the chelator sodium citrate. This results in sodium alginate fibers with a significantly higher swelling ratio than calcium alginate fibers.^[32] As in CA, the alginate strands in TSC can be stabilized by hydrogen bonds, which explains the comparable values of the rate k_2 for 0 and 1% MC content. However, at 1% MC in the fiber, the value of k_2 increases to that of GDL (blue dashed line). This can be explained by the strength of the H-bridges, which are significantly weaker in TSC (pH 8) compared to CA at pH 2 due to the lower proton concentration.

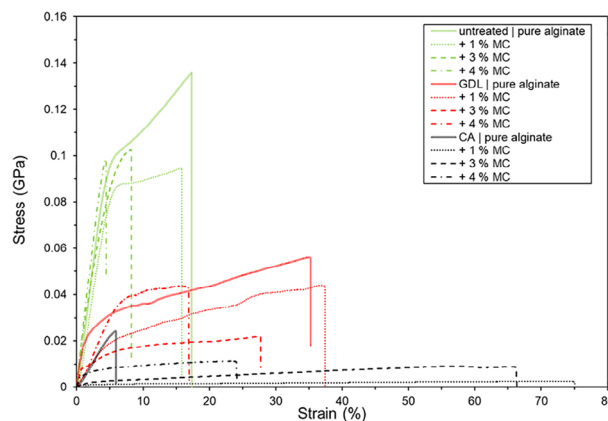


Figure 4. Tensile tests on single alginate-casein fibers with different MC contents pre-treated in different solutions (CA and GDL) compared to reference samples.

A similar trend can be seen for the transition time t' . The values at 0% and 0.5% MC content are comparable to those of CA, but larger than those of GDL, due to the stabilization of the sodium-alginate chains by hydrogen bonds. From 1% MC, the transition time increases rapidly, reaching the final value of $t' = 100$ s at 2% MC (Figure 3f).

The shift of the transition to longer times can be explained by the calcium supply within the MC, which is less released from the micelle than in the acidic solutions. This delays the transition from calcium alginate to calcium depleted alginate fiber. In contrast to the swelling in CA and GDL, the rate of transition in TSC increases with increasing MC content. This can be explained by a homogeneously swollen structure allowing a more concerted exchange of calcium for sodium within the fiber (see Figure S4c2, Supporting Information).

2.3. Mechanical Properties of the Fibers

In order to investigate the effect of treatment with acidic solvents on the functional properties of the fibers, the mechanical properties were examined in comparison with untreated samples. Prior to the mechanical tests, the fibers were treated for one hour in 1 M GDL and separately in 1 M CA. Afterwards they were dried on a rack for 3–4 h. Fibers treated in 1 M TSC could not be tested as some of them could not be harvested non-destructively after only 10 min in the TSC due to severe swelling. **Figure 4** shows representative individual measurements from the tensile tests in direct comparison. It can be seen that the fibers have a reduced elasticity due to the treatment in the acidic solutions. This manifests itself in a greater elongation for a given tensile stress and an earlier transition from the linear elastic to the plastic range. In addition, both fracture and plastic deformation occur at lower stresses and higher strains. This effect is particularly evident in the measurements on fibers treated with CA (black lines). Treatment with GDL (red lines) also resulted in greater elongation at low stress, but not as much as with CA.

The casein content in the fiber also influences the stress-strain curves. As the MC content increases, the fibers appear to become

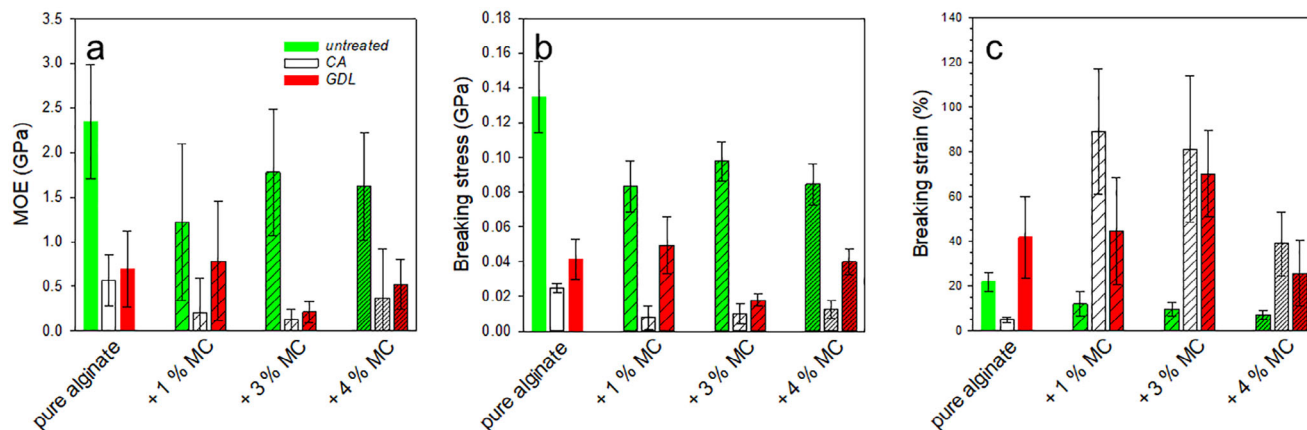


Figure 5. MOE a), breaking stress b), and breaking strain c) of tensile tests on fibers with different MC contents. For comparison, the values for the untreated sample and the effect of prior treatment in acid solutions (CA and GDL) are shown.

more brittle. This manifests itself in a lower elongation at a given stress and an earlier failure of the fibers (broken lines).

For a more detailed analysis, the Young's modulus of elasticity (MOE), breaking stress, and breaking strain were determined from the stress-strain curves. The corresponding values are shown in **Figure 5** in the form of a bar chart with different colors (depending on the treatment) and shading (depending on the MC content in the fiber). The error bars shown are the result of the five measurements made for each test condition.

The average MOE of the untreated sample ranges from 1.2 to 2.4 GPa with no clear trend as a function of MC content in the fiber. However, there is a clear trend towards lower MOE values when the fibers are treated with the acidic solutions GDL and CA prior to measurement. This effect is particularly evident in the alginate fiber with 3% MC content. Treatment with GDL reduces the MOE from 1.77 to 0.21 GPa ($p = 0.0012$) and with CA to 0.124 GPa ($p = 0.0009$). However, the fact that the effect at 4% MC is not visible due to the variability in the data could be related to a partial loss of internal fiber structuring, as seen in the SEM image (Figure S1c, Supporting Information). The loss of elasticity can be explained by the acid-induced dissolution of the stabilizing ionic calcium contacts between the alginate chains.^[29] In comparison, the MOE values of the pure alginate fiber are significantly higher after acid treatment. This indicates that the MC-alginate interactions weaken the cohesion between the alginate chains. This can also be seen in the lower breaking stress values for the MC-alginate composite fibers (untreated and CA treated) compared to the pure alginate fibers (Figure 5b). However, a dependence of the breaking stress on the MC content in the fiber cannot be deduced from the data. The mean breaking stress values for the untreated composite fibers vary between 0.083 and 0.097 GPa. However, a significant reduction in the values can be observed as a result of the acid treatment. After treatment in CA, the values are between 0.0078 and 0.02 GPa, i.e., about 1/10 of the value of the untreated sample.

After GDL treatment, the breaking stress for the 3% MC composite fibers fell particularly sharply from 0.097 to 0.018 GPa ($p = 0$). The reduced breaking stress after acid treatment of the fibers correlates with the reduced MOE values (Figure 5a). Reduced MOE values due to calcium removal from the alginate by acid

treatment could lead to earlier plastic deformation and ultimately failure at lower loads.

The breaking strain values in Figure 5c clearly show that the fibers become much more ductile after acid treatment. Even for fibers with only 1% MC content, CA treatment leads to an increase in elongation at break from 11.7% to 89%. ($p = 0.0003$). Treatment with GDL also had an effect on breaking strain, but not as great as with CA when considered over all fibers. However, in the case of composite fibers containing, for example, 3% MC, the breaking strain increased from 9.6% to 70.2% ($p = 0.0001$). Whereas for the untreated fibers there is a tendency for the breaking strain to decrease with increasing casein content, after CA treatment the elongation at break for fibers with 1 and 2% MC content increases sharply, before decreasing slightly at 3% MC to a level that is still significantly higher than that of the pure alginate fiber. In contrast, no dependence on MC content can be detected for fibers after GDL treatment.

3. Conclusion

In this study, the swelling behavior and mechanical stability of alginate fibers with different MC contents were investigated in different solutions. These solutions weakened the stabilizing calcium contacts within the fibers either by high proton concentrations or by chelating agents. Compared to simple hydration in water, swelling in the decalcifying solutions occurred in two phases: by solvent uptake and by further expansion due to destabilization of the calcium cross-links. An extended Schott model was used to describe the swelling rates and the total swelling capacity of each process, as well as the transition from one phase to the other, which provides information on the homogeneity of the swelling.

A comparison of the different decalcification methods showed that TSC at pH 8 caused a slower but significantly stronger swelling of the composite fibers than CA and GDL. However, the composite fibers remained stable in the organic acids and could be harvested, which is not the case for MC fibers without alginate as reported in the literature.

The mechanical properties of the fibers changed as a result of CA and GDL treatment. On the one hand, all treated samples became softer and less stable, which was reflected in reduced values

for MOE and breaking stress. On the other hand, the elongation at break also increased. This effect is particularly evident in composite fibers with 1 or 3% MC content if they were previously treated with CA. The reason for this is the stabilization of the fiber structure by hydrogen bonding, which makes the fibers even more flexible compared to the GDL treatment. The study provides a basis for the development of calcium exchange processes for the production of composite fibers with adaptable mechanical properties and more stable structures for industrial applications.

4. Experimental Section

Materials: MC was extracted in its natural form from raw milk. The milk was sourced from a local dairy farm in Aachen and processed on the same day. The milk was centrifuged for 20 min at 3000 RCF (Hettich centrifuge, Germany) to remove the fat from the milk. The milk was then centrifuged in an ultracentrifuge (Beckman Coulter, Brea, USA) at 70 000 RCF for 1 h to separate the casein micelles from the whey proteins in the milk. For resuspension, the casein micelle pellets were resuspended in simulated milk ultra-filtrate, SMUF solution (prepared according to Dümpler et al. [39]) and stirred at 37 °C for 2 h. To prevent the growth of microbes in the sample, 0.5 g L⁻¹ sodium azide (99% Carl Roth, Karlsruhe) was also added. Finally, a 10% casein solution was prepared. The casein micelles exhibited a size distribution between 150 and 600 nm as determined by dynamic light scattering and shown in Figure S5 (Supporting Information). To prepare a 6% sodium alginate solution, sodium alginate powder (Carl Roth, Karlsruhe, Germany) was dissolved in water with stirring for 4 h at 40 °C to ensure that the alginate was completely hydrated in the water and a homogeneous solution was formed.

Fiber Production: Starting solutions for fiber production with 0%, 0.5%, 1%, 2%, 3%, and 4% (w/w) casein content and 3% (w/w) alginate content were prepared by mixing SMUF buffer or casein and alginate solutions with stirring for 3 min. The alginate was dissolved in deionized water prior to preparation. For the coagulation bath, calcium chloride powder (Carl Roth, Karlsruhe, Germany) was dissolved in de-ionized water to obtain a solution with a concentration of 0.1 M. The coagulation bath was maintained at a temperature of 60 °C. The total volume of the coagulation bath was 1 L and was used for the production of no more than 5 fibers. The mixture was extruded using a laboratory syringe pump (Fusion 4000, Chemyx Inc., Stafford, USA) connected by a tube to a dosing needle (Vieweg, Germany) with an internal diameter of 0.8 mm. The flow rate of the syringe pump was set at a constant 0.4 mL min⁻¹ to ensure uniform fiber formation. Fiber formation occurred after the mixture of casein and sodium alginate came into contact with calcium chloride from the coagulation bath. A representative image of this fiber formation process in the coagulation bath is shown in Figure S6 (Supporting Information).

Immediately afterwards, the fibers, which were originally about 20 cm long, were removed from the coagulation bath with small tweezers and hung on a rack to dry. This ensured that the fibers dried in the air in a cylindrical shape without touching any surfaces. The drying process took about 3–4 h. After drying, the fibers were cut into smaller pieces of about 5 cm in length for the subsequent experiments.

Swelling Experiment and Modelling: For the swelling tests, 10 mL of the appropriate swelling medium was added to the fiber pieces placed inside a petri dish with a diameter of 5 cm. The increase in fiber width was then monitored using an optical microscope (Nikon, Japan). For each swelling process, a video was recorded for 600 s using a digital camera (AMscope, USA). The change in fiber diameter over time was evaluated from extracted photographs using ImageJ software (NIH, USA). To obtain an average value, the swelling experiments were repeated on three separate fibers.

The increase in fiber width was determined using

$$\text{Width increase (\%)} = \left(\frac{d - d_0}{d_0} \right) \cdot 100 \quad (1)$$

where d_0 is the original width of the dried fiber and d is the width of the hydrated fibre determined at time t . Deionised water, 1 M CA monohydrate solution in water (VWR Chemicals, Germany), 1 M GDL in water (Thermo Scientific Chemicals, USA) and 1 M TSC in water (Acros Organics, Belgium) were used as swelling media.

The Schott model was used to analyze the swelling kinetics in water, where the assumption of an initially dry fiber corresponds to a special case of the model with zero initial moisture content, leading to the following equation:[40]

$$S = \frac{k_s \cdot S_\infty^2 \cdot t}{1 + k_s \cdot S_\infty \cdot t} \quad (2)$$

where S is the solvent uptake at time t , k_s is the specific swelling rate and S_∞ is the swelling value reached at time $t \rightarrow \infty$.

An extended Schott model was used to analyze the swelling kinetics in the decalcifying media,[13] which can be specified in differential form:

$$\frac{dS}{dt} = w_1 \cdot k_1 \cdot (S_{1,\infty} - S)^2 + w_2 \cdot k_2 \cdot (S_{2,\infty} - S)^2 \quad (3)$$

here, the swelling rate dS/dt is composed of two simple swelling processes according to Equation 2, with respective specific swelling rates and theoretical swelling values at infinity. Both processes are coupled by a time-dependent transition functions:

$$w_1 = \left(\frac{1}{1 + g(t)} \right), \quad w_2 = \left(\frac{g(t)}{1 + g(t)} \right) \quad (4)$$

defined by an exponential function:

$$g(t) = e^{(t-t') \cdot k_t} \quad (5)$$

with rate k_t and delay time t' .

The second order swelling equations were fitted to the experimental swelling data using GNU Octave software (free ware, USA).

Mechanical Stress Tests: Fibers treated with either 1 M CA or 1 M GDL for one hour after manufacture or untreated fibers were used for mechanical testing. For treatment, 10 mL of the acid solution was first added to a petri dish, and then fiber pieces of the sample were submerged in it. Mechanical tests were always performed on dried fibers using a texture analyzer (Shimadzu, AGS-X, Kyoto, Japan). The fiber diameter was measured beforehand using a light microscope. The fibers were glued to a plastic template to ensure that all fibers had a length of 10 mm. After four hours, the fibers were placed in the texture analyzer for testing. The sample was then pulled at a speed of 50 microns per second until it broke. During the measurement, the force and displacement were measured by the texture analyzer.

All tests were repeated five times. The MOE was determined at strains between 0.3% and 1%. The breaking stress and elongation at break were determined from the stress-strain curves obtained. The mean values and standard deviations of the material properties were calculated from the sample sizes. Two-way ANOVA analysis was performed using Octave software to assess the significance of MOE, breaking stress, and breaking strain.

Confocal Fluorescence Microscopy: Composite fibers of MC and alginate were stained by allowing 0.05% w/w 3-hydroxypyrene-1,3,6-trisulphonic acid (Fluka) to diffuse into the fibers, selectively labeling the embedded casein micelles. Individual fibers were immersed for 10 min in separately prepared fresh dye solutions, then rinsed in water to remove excess dye and subsequently air-dried. An inverted confocal laser scanning microscope (LSM 980 with Airyscan 2, Carl Zeiss AG, Oberkochen, Germany) was used for microscopic analysis. To record the fluorescence signal of the stained structures, the sample was irradiated with a laser with an excitation wavelength of 405 nm and emission was recorded at 411 and 606 nm. Both the fluorescence signal and the brightfield image

were recorded using a 32-channel GaAsP detector. All images were stored with a resolution of 2294×2294 pixels and a bit depth of 16 bits per pixel.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

alginate composites, fibers, kinetic modelling, micellar casein, swelling

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