



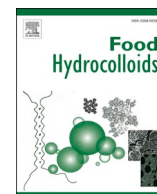
Elucidating the contributions of alginate and fucoidan to algal cell wall structure and rheology using bacterial cellulose gels

Downloaded from: <https://research.chalmers.se>, 2026-10-13 05:44 UTC

Citation for the original published paper (version of record):

Cazón, P., Area, M., Crespo, A. et al (2027). Elucidating the contributions of alginate and fucoidan to algal cell wall structure and rheology using bacterial cellulose gels. Food Hydrocolloids, 185. <http://dx.doi.org/10.1016/j.foodhyd.2026.113430>

N.B. When citing this work, cite the original published paper.



Elucidating the contributions of alginate and fucoidan to algal cell wall structure and rheology using bacterial cellulose gels

Patricia Cazón^{a,b}, Marta Area^c, Andrea Crespo^c, Jesus Valcarcel^c,
Maria Teresa Ahumada Ochoa^d, Mathias Johansson^d, Jummi Laishram^e, Regina Ciano^{e,f},
Anna Ström^d, Patricia Lopez-Sanchez^{c,*}

^a Department of Chemical Engineering, Facultade de Ciencias, Universidade de Santiago de Compostela, Campus Terra, Lugo, 27002, Spain

^b Dairy and Food Technology Centre (APTLA), Universidade de Santiago de Compostela, Rua Montiron 150, Lugo, 27002, Spain

^c Department of Food Technology, Instituto de Investigaciones Marinas-Centro Superior de Investigaciones Científicas (IIM-CSIC), Rúa de Eduardo Cabello 6, Vigo, 36208, Spain

^d Applied Chemistry, Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Gothenburg, 412 96, Sweden

^e Area Science Park, Padriciano 99, Trieste, 34149, Italy

^f CNR-IOM, Strada Statale 14, Basovizza, Trieste, 34149, Italy

ARTICLE INFO

Keywords:

Bacterial cellulose
Alginate
Fucoidan
Algae
Rheology
Gels
Processing

ABSTRACT

Bacterial cellulose gels (BC) containing alginate or fucoidan were developed to investigate cellulose-algal polysaccharide interactions and, their impact on gel structure, mechanics, rheology and processing stability. Alginate, a well-established thickening polysaccharide in the food industry, and fucoidan, a sulphated polysaccharide with emerging applications due to its bioactive potential, were incorporated into the incubation medium during cellulose synthesis. A multiscale characterisation approach combining solid-state ¹³C NMR, X-ray diffraction, scanning electron microscopy (SEM), rheological and mechanical analyses was employed to elucidate the organisation and interactions within the gel network. The presence of either polysaccharide led to a reduced elastic modulus *G'* of the gels, likely reflecting a reduction in cellulose fibril entanglement density, and was associated with lower compressive stress values. BC gels containing alginate exhibited complete stress relaxation following compression, whereas BC gels retained residual compressive stress, indicating a reduced capacity for stress dissipation in BC networks. Both alginate and fucoidan were located in close proximity to cellulose fibres, with fucoidan appearing to coat the fibre surfaces. Neither polysaccharide altered cellulose crystallinity, and both behaved as mobile components within the gels. After dehydration, the gels did not fully rehydrate, regardless of the presence of non-cellulosic polysaccharides. Following thermal treatment in water, alginate was solubilised from the gels, whereas fucoidan remained within the gel network. The results revealed distinct roles of alginate and fucoidan in modulating bacterial cellulose network assembly, viscoelasticity, and mechanical performance. This study provides new insights into cellulose-marine polysaccharide interactions relevant to better understanding algal cell walls and demonstrates the potential of bacterial cellulose gels as tuneable biomaterials for technological applications.

1. Introduction

Macroalgal polysaccharides are widely used in the food industry as thickening, gelling, and texturising agents due to their physicochemical and rheological properties (Kraithong et al., 2025). Among these biopolymers, alginates stand out due to their unique gelling properties and versatility in food applications. Alginates are linear anionic

heteropolysaccharides composed of β -D-mannuronic acid (M) and α -L-guluronic acid (G) residues linked through (1 $\rightarrow$ 4) glycosidic bonds. Their molecular weight, together with the distribution of M and G units, strongly influences their physicochemical behaviour, including viscosity, gelation, stiffness, and elasticity (Draget & Taylor, 2011; Skjakbraek et al., 1986). In brown algae, alginates may account for up to 40% of the dry biomass and are located in the cell wall and extracellular matrix,

This article is part of a special issue entitled: 20th Food Colloids published in Food Hydrocolloids.

* Corresponding author.

E-mail address: plopez@iim.csic.es (P. Lopez-Sanchez).

<https://doi.org/10.1016/j.foodhyd.2026.113430>

Received 3 July 2026; Received in revised form 12 September 2026; Accepted 22 September 2026

Available online 24 September 2026

0268-005X/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

where they act as a primary structural polysaccharide (Mazéas et al., 2023). Fucoidan is another major polysaccharide present in brown algae cell walls and intercellular spaces. It is a sulphated heteropolysaccharide mainly composed of fucose residues linked by α -(1 $\rightarrow$ 3) glycosidic bonds or alternating α -(1 $\rightarrow$ 3)/ α -(1 $\rightarrow$ 4) linkages, although other monosaccharides, including galactose, glucose, mannose, xylose, rhamnose, and uronic acids, may also be present depending on the algal species (Bilan et al., 2010; Palanisamy et al., 2017). Fucoidan generally represents 5–10% of the dry biomass and plays important structural and protective roles against desiccation and environmental stress (Deniaud-Bouët et al., 2014). In addition, fucoidan has attracted increasing attention due to its reported biological activities and potential applications in functional foods and biomaterials (Kopplin et al., 2018; Palanisamy et al., 2017; Yuk Ko et al., 2026). Cellulose consists of linear chains of glucose residues linked by β -(1 $\rightarrow$ 4) glycosidic bonds. Extensive hydrogen bonding and van der Waals interactions between cellulose chains promote the formation of highly ordered nanofibrils, which further assemble into microfibrils containing alternating crystalline and amorphous regions. Although cellulose is present in green, red, and brown macroalgae, its abundance varies significantly among taxa (Baghel et al., 2021). In brown algae, cellulose typically accounts for 2–10% of the dry biomass (Baghel et al., 2021). Despite the importance of brown algal cell wall polysaccharides, studies elucidating the interactions governing their structural organisation and role in the mechanical behaviour of algae are scarce (Deniaud-Bouët et al., 2014). A deeper understanding of these interactions is essential because the cell wall acts as a structural barrier that modulates the accessibility and extraction of bioactive compounds from algal biomass (Perez-Roa et al., 2025; Souto-Prieto et al., 2025; Vega-Gomez et al., 2026). Elucidating the relationship between cell wall composition and physicochemical properties could contribute to the optimisation of extraction processes, improvement of nutritional functionality, and development of novel algae-derived biomaterials.

Due to the complexity of cell walls, simplified model systems based on bacterial cellulose (BC) gels have been employed to investigate the role of plant polysaccharides and their interactions with cellulose (Lopez-Sanchez et al., 2015, 2016; Mikkelsen et al., 2015; Whitney et al., 1995). These gels mimic key aspects of natural cell wall assembly, as cellulose fibrils are synthesised and deposited in the presence of non-cellulosic polysaccharides, analogous to cellulose deposition within the extracellular matrix of plant and algal tissues (Whitney et al., 1999). BC is suitable for such studies due to its high purity, elevated crystallinity, and ability to form interconnected three-dimensional fibrillar networks, enabling controlled investigations of polysaccharide interactions and mechanical and rheological behaviour of polysaccharide-rich matrices. Previous studies have demonstrated that BC gels mechanical performance emerges from the coupling between network elasticity and fluid dynamics (Lopez-Sanchez et al., 2014), a phenomenon particularly relevant to biological tissues, where deformation and mechanical resistance are highly dependent on matrix hydration and polysaccharide interactions. Although bacterial cellulose-based networks have previously been used as model systems to investigate plant cell walls, their application to algal cell walls remains unexplored. In this context, we synthesised BC-based gels as a simplified model to elucidate the contribution of alginate and fucoidan to the structure, mechanics, and rheological behaviour of algal cell walls. BC-alginate gels have been investigated as sustainable packaging materials and for biomedical applications (Gromovkyh et al., 2025; Kanjanamosit et al., 2010). The novelty of our study lies on the use of a BC three-dimensional controlled system for disentangle the contributions of individual algal polysaccharides, to gain insights into structure-rheology relationships relevant to brown algal cell walls properties. Beyond improving our understanding of algal cell wall architecture, this biomimetic approach may support the rational design of nature-inspired biomaterials with potential applications in food and biotechnology.

2. Materials and methods

2.1. Synthesis of bacterial cellulose gels

To obtain the BC gels, the bacterium *Komagataeibacter hansenii* strain ATCC® 53582 (Manassas, VA, USA) was incubated in Hestrin-Schramm (HS) medium under static conditions at 30 °C for 72h in 34 mm diameter containers following the method earlier described by (Mikkelsen et al., 2020) at pH 5 with a glucose concentration of 2% w/v. Two types of polysaccharides were studied: i) alginate (CEAMTEX 1602, Ceamsa, Pontevedra, Spain) and ii) fucoidan (FCSL50, Oceanium, Oban, UK) with a composition of 80% fucoidan, 15% protein and 5% alginate. Prior to inoculation, individual polysaccharide solutions were added to the HS incubation medium to reach a final concentration of 0.5% w/v, this concentration was selected based on previous studies (Lopez-Sanchez et al., 2016). After the incubation period, the pellicle that forms at the liquid–air interface is carefully collected and washed six times with ice-cold water under agitation at 50 rpm in the case of BC samples, or with a 0.5% (w/v) aqueous solution of alginate or fucoidan in the case of BC-alginate or BC-fucoidan samples. The purpose of this approach was to minimise the concentration gradient between the gel and the external washing medium and thereby reduce passive diffusion/leaching of the incorporated polysaccharide during washing. In this way, the HS medium and remaining bacterial cells are removed, while the polysaccharide remains retained in the matrix. All gels were stored in a 0.02% NaN₃ solution or in the corresponding polysaccharide solution (0.5% w/v) at 4 °C until further analysis.

2.2. Processing of bacterial cellulose gels

2.2.1. Thermal treatment at 90 °C (TT90)

A glass beaker was filled with 1 L of Milli-Q water and heated to 90 °C. Once this temperature was reached, the gels were placed in the beaker, which was then covered with aluminium foil to reduce water evaporation, and heated while stirring at 300 rpm for 30 min. The gels were weighed before and after treatment. Ten replicates were analysed.

2.2.2. Ultrasound (US)

The gels were treated in a temperature-controlled ultrasonic bath (Ultrasons HD, JP Selecta, Spain) at 40 kHz for 30 min at 20 °C. The gels were placed in a beaker containing 1L of Milli-Q water, and the beaker was then placed in the bath. The gels were weighed before and after treatment. Ten replicates were analysed.

2.2.3. Freeze-drying and rehydration

Gels were frozen at –80 °C for 24 h prior to freeze-drying. Samples were then freeze-dried for 48 h (Telstar LyoAlfa Plus 15-85, Barcelona, Spain). The weight of the gels was recorded before and after drying. Rehydration was performed by immersing the samples in 1 L of distilled water at room temperature for 24 h, after which the samples were weighed again. Ten replicates were analysed.

2.3. Dry weight measurement

Gels were dried in an oven at 105 °C for 24 h. The dry matter content was calculated in triplicate by weighing the gels before and after drying.

2.4. Monosaccharide analysis

Freeze-dried samples were cut first with scissors and further cut into fine pieces using a scalpel prior to analysis. Approximately 5 mg of freeze-dried sample was weighed into a clean glass tube fitted with a Teflon-lined screw cap. Subsequently, 250 μ L of 12 M H₂SO₄ was added, and the samples were incubated at room temperature for 1 h with intermittent vortex mixing. Complete dissolution of the sample, indicated by the development of a dark brown to black coloration, was

verified before proceeding; when necessary, incubation time was extended up to 3 h. The acid was then diluted by adding 2.75 mL of distilled water, and the hydrolysis was continued by incubating the tightly capped tubes in an oven at 100 °C for 3 h. Monosaccharides were measured using high-performance liquid chromatography (HPLC) on a Jasco chromatographic system (Jasco Inc., Tokyo, Japan) equipped with an AS-2050 Plus Intelligent Sampler autosampler, a PU-2089 Plus high-pressure pump, a CO-2065 Plus column oven, and an RI-2031 Plus refractive index detector. Separation was performed on a Rezex RHM-Monosaccharide H+ (8%) column using 5 mM H₂SO₄ as the mobile phase. The eluent was filtered through 0.45 µm nylon membrane filters before use. Prior to analysis, the column was conditioned with the mobile phase at a flow rate of 0.04 mL min⁻¹ for several hours. Isocratic elution was subsequently carried out at a flow rate of 0.4 mL min⁻¹ and a column temperature of 45 °C. Glucose and fucose were identified and quantified using external calibration curves prepared from the analytical standards D-glucose and L-fucose (Sigma-Aldrich, Massachusetts, United States). Standard solutions were prepared at a concentration of 1 g L⁻¹ to determine retention times and establish calibration relationships between peak area and analyte concentration. Chromatographic data acquisition and analysis were performed using EZChrom software (version 3.2.6). Samples were measured in duplicates.

2.5. Uronic acid assay

Total uronic acid was measured following the method by (Filisetti-Cozzi & Carpita, 1991) using m-hydroxybiphenyl. Approximately 10 mg of sample were hydrolysed in 2.4 mL of 12.5 mM sodium tetraborate in H₂SO₄ with the addition of 40 µL of 4 M potassium sulfamate (pH 1.6) to prevent the interference caused by browning that occurs during sample hydrolysis. The mixture was then incubated for 20 min in an oven at 95 °C and cooled on ice. Subsequently, the samples were diluted 1:10 with Milli-Q water to a final volume of 3 mL. A volume of 80 µL of 3-phenylphenol were added to react with the released uronic acids. The mixture was thoroughly mixed and allowed to react for 10 s prior to UV-Vis spectrometric measurement (UV-1700 PharmaSpec, Shimadzu, Japan) at 525 nm. The total uronic acids were calculated based on calibration with alginate solutions (0, 40, 80, 160, 200 µg/mL). Samples were measured in duplicates.

2.6. Molar mass

Molar mass was determined on a GPC/SEC system (Agilent 1260, Agilent, Waldbronn, Germany) equipped with quaternary pump (G1311B), injector (G1329B), column oven (G1316A), refractive index (G1362A) and dual angle light scattering (G7800A) detectors. Sample separation was achieved with a set of three Agilent columns: Suprema precolumn (5 µm, 8 × 50 mm), Suprema 30 Å (5 µm, 8 × 300 mm), and Suprema ultrahigh (10 µm, 8 × 300 mm). A sample volume of 100 µL was injected onto the above system and eluted isocratically at 1 mL/min with a mobile phase solution composed of 0.1 M NaNO₃ and 0.01 M NaH₂PO₄ at pH 6.6. The column was kept at 30 °C, and the detectors at 40 °C. Both detectors were calibrated with a polyethylene oxide standard (Agilent) of 101.8 kDa (Mp) and a polydispersity index (PDI) of 1.07. Samples and standards were dissolved in the mobile phase solution. Refractive index increments (dn/dc) of 0.150 for alginate (Turquois & Gloria, 2000) and 0.115 for fucoidan (Kopplin et al., 2018) were used for the calculations. Samples were measured in triplicates.

2.7. ζ potential measurement

The ζ potential of alginate and fucoidan was measured using a Litesizer 500 (Anton Paar, Anton Paar GmbH, Graz, Austria). Polysaccharides were dispersed in 10 mM NaCl to a final concentration of 0.1% w/v. Measurements were performed using an omega cuvette (Anton Paar GmbH, Graz, Austria). All samples were measured in

triplicate with 100 runs.

2.8. Shear viscosity of alginate and fucoidan

Alginate and fucoidan aqueous solutions (0.5% w/v) were prepared by dispersing the polysaccharides in 100 mM NaCl. Shear viscosity measurements were performed using a MCR 302 rheometer (Anton Paar GmbH, Graz, Austria), equipped with a plate-plate geometry and a gap of 500 µm. Steady state measurements at 25 °C were carried out with logarithmically increasing shear rate from 0.1 to 100 s⁻¹. Samples were measured in triplicates.

2.9. ¹H solution-state nuclear magnetic resonance (NMR)

The chemical characterisation of alginate include the determination of L-gulonate (G) and D-mannuronate (M), following previously described methods (Grasdalen et al., 1981; Salomonsen et al., 2009) with some modifications. In brief, the samples were partly degraded by mild hydrolysis in HCl at pH 3 and 90 °C for 30 min. Samples were dried in an oven at 70 °C overnight and, dissolved in D₂O (1% (w/v) at pH 7. ¹H NMR spectra were recorded using a 400 MHz Bruker Avance NEO system spectrometer (Bruker, Massachusetts, USA) using 8k data-points, spectral widths of 5 kHz (25 MHz) or 10 kHz (50 MHz), a 75° pulse, a pulse repetition time of 0.8 s, and, typically, 40,000–50,000 scans. Spectra was recorded at 84.9 °C. Peak areas were measured by planimetry. For the lines of C-1, C-2(G), C-6, C-4(M), and C-5(M), the overlap of adjacent peaks was visually estimated. Data processing was done in MestReNova. Two independent samples were measured.

2.10. Solid state nuclear magnetic resonance (NMR)

¹H-¹³C cross-polarization magic-angle spinning CP/MAS NMR spectra were recorded on a 600 MHz Varian spectrometer using a 3.2 mm HX CP-MAS probe. Samples were packed into 3.2 mm zirconia rotors and spun at 5 kHz. ¹H excitation was achieved using a 2.5 µs 90° pulse. Cross polarization was performed with a 1.0 ms contact time using a linear amplitude ramp on the ¹H channel and high-power ¹H decoupling during acquisition. A total of 5000 transients were accumulated with a recycle delay of 3.0 s. ¹³C chemical shifts were referenced to tetramethylsilane (TMS) using an external reference, corresponding to a ¹³C reference frequency of 150.79 MHz. Two independent samples were measured.

2.11. Fourier transform infrared spectroscopy (FTIR)

FT-IR was carried out using an FT/IR-4X spectrophotometer (Jasco Inc., Hachioji, Tokyo, Japan) equipped with an ATR-PRO 4X accessory incorporating a high-performance monolithic diamond crystal. Spectra were acquired in the wavenumber range of 4000–400 cm⁻¹ at a spectral resolution of 4 cm⁻¹ and processed using Spectra Manager™ software. Prior to the test, the selected samples were dried in an oven at 105 °C for 24 h. Samples were conditioned at 57 ± 2% relative humidity and 30 ± 1 °C for 48 h. FTIR spectra were recorded at a spectral resolution of 4 cm⁻¹ in the range 400–4000 cm⁻¹. Spectra visualization was carried out using Spectragryph software (version 1.2.16.1). Two independent samples were measured.

2.12. X-ray diffraction (XRD)

To avoid the broad background water signal, samples were air dried at room temperature prior to analysis. X-ray diffraction of dried samples was performed using a Bruker D8 Discover instrument with a Bragg-Brentano setup with a copper X-ray generator (Kα radiation source, λ = 1.54 Å), covering 2θ over the angular range 10°–60°. A single crystal Si holder was used. Data was analysed using DIFFRAC.EVA software (version 7.3). A Gaussian function was used to fit the diffraction profiles.

The crystallite sizes were estimated from the three different lattice planes of cellulose I α and I β using the Sherrer equation (equation (1)).

$$L_{(hkl)} = \frac{k\lambda}{B_{(hkl)} \cos \theta} \quad (\text{Equation 1})$$

where ($L_{(hkl)}$) is the size of the crystallite (nm), k is the Scherrer constant (0.94), λ is the X-ray wavelength, $B_{(hkl)}$ is the full-width at half-maximum of the reflection hkl and 2θ is the corresponding Bragg angle.

The approximate crystallinity index (CI%) was calculated as described in equation (2).

$$CI = \frac{(I_c - I_a)}{I_c} \quad (\text{Equation 2})$$

where I_c is the intensity of the crystalline peak and I_a is the intensity of the amorphous region. Two independent samples were measured.

2.13. Scanning electron microscopy (SEM)

Samples were cut into approximately 1 cm² pieces, frozen in liquid nitrogen for 10 s and subsequently prepared using a critical point dryer (BAL-TEC CPD 030, Balzers, Liechtenstein) following a series of dehydration steps as described in Lopez-Sanchez et al. (Lopez-Sanchez et al., 2015). Scanning electron microscopy imaging was performed using a FIB-SEM Tescan Amber X (Tescan, Brno, Czech Republic) dual-beam system, equipped with a xenon (Xe) plasma ion source. Samples were mounted on aluminium stubs using carbon adhesive tape to ensure proper electrical grounding. To mitigate charging effects associated with the insulating cellulose matrix, specimens were sputter-coated with a ~20 nm thick gold layer using a Leica EM ACE 600 operating under high vacuum conditions, providing a uniform, fine-grained conductive coating. SEM imaging was then carried out at a landing energy of 2 keV and a beam current of 300 pA, with a working distance of 6 mm. These conditions were selected to minimise the electron interaction volume and probe size, thereby enhancing surface sensitivity and spatial resolution. Secondary electron (SE) images were acquired using an Everhart-Thornley detector (ETD). Low-magnification overview images were initially acquired to identify representative regions of interest, followed by higher magnification imaging to resolve surface morphology at different length scales. Images were taken from at least three different locations of each sample and 6 images were taken from each position. All images were taken from the top side of the sample, which is the one in contact with air during cellulose production.

2.14. Mechanical testing and oscillatory rheology

Gels were measured on a rheometer MCR 302 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a Peltier temperature control (P-PTD220). A 40 mm diameter plate (calibrated for 34 mm diameter samples) was used. Both the upper and bottom plates were coated with fine emery paper to reduce sample slip during measurements (Lopez-Sanchez et al., 2014). Gels were placed in the centre of the bottom plate in the rheometer and, for each experiment, the gap was adjusted according to a normal force of 0.4N corresponding to the sample thickness (Lopez-Sanchez et al., 2014). Axial compression was applied at a constant speed of 100 $\mu\text{m/s}$ for 250 μm whilst measuring the normal force. From the normal force values the compression stress was calculated after setting the initial normal force to 0. After the compression step, the relaxation of the normal force was followed simultaneously with a small amplitude oscillatory deformation test carried out for 180 s at a frequency of 0.016 rad/s (1 Hz) and at a constant stress of 1 Pa, chosen from the linear viscoelastic regime. Cycles of three compressions and three oscillations were performed. Measurements were carried out at 25 °C. For each type of gel three samples were measured. The weight, thickness and diameters of the samples were recorded before and after the test.

2.15. Statistical analysis

One-way ANOVA was employed to evaluate the differences in composition, rheology and mechanics. Significant differences between mean values were obtained through pair-wise comparisons using Tukey's PostHoc test (confidence interval of 95%).

3. Results and discussion

3.1. Characterisation of alginate and fucoidan

The weight-average molecular weight (M_w) of alginate was 343 kDa (Table 1), in the same range as previously reported values (Draget et al., 1996). The G:M ratio was 0.17, as determined by ¹H NMR. Alginate is a negatively charged polymer; in this case, it exhibited a zeta potential of -40 mV, indicating good colloidal stability. In comparison, fucoidan exhibited a higher molecular weight of 1038 kDa and a ζ potential of -25 mV. Fucoidan is a highly heterogeneous sulphated polysaccharide with a very broad range of reported molecular weights depending on the extraction method (Morimoto et al., 2014). Both polymers showed similar polydispersity (M_w/M_n). At the same concentration, alginate displayed higher viscosity than fucoidan, with values of 81.6 mPa s and 2.3 mPa s, respectively.

3.2. Composition of bacterial cellulose gels

All gels were disks in shape with thickness that vary between 1.97 and 2.74 mm (Table 2). BC_{Alginate} exhibited a significant higher wet weight compared to BC, whereas BC_{Fucoidan} showed a significant lower wet weight (Table 2). The higher wet weight of BC_{Alginate} gels may be attributed to higher cellulose deposition and incorporation of alginate, as reflected by the increased dry weight, and to enhanced water retention due to the hydrophilic properties of alginate. Since the increase in dry weight alone shown in Table 2 is not sufficient to fully account for the observed rise in wet weight, it is proposed that the higher wet mass results from a combination of greater solid content and an increased water-binding capacity compared to cellulose alone. BC_{Fucoidan} exhibited a dry weight similar to BC (22-24 mg), therefore the lower wet weight observed for BC_{Fucoidan} suggests a reduced water-holding capacity compared to both BC and BC_{Alginate}, due to differences in network structure, such as increased crosslinking density *i.e.* number of entanglements between cellulose fibres, or reduced availability of hydrophilic sites, which limit water retention. Although fucoidan is also a hydrophilic polysaccharide, its interaction with cellulose fibres may lead to partial masking of cellulose hydroxyl groups and the formation of a more compact network structure. This can reduce the accessibility of water-binding sites and limit water uptake, resulting in a lower wet weight despite similar dry mass and density. The density of the gels was 0.012-0.016 g/cm³, densities of 0.007-0.017 g/cm³ have been previously reported for bacterial cellulose and bacterial cellulose gels with hemicelluloses (Lopez-Sanchez et al., 2015). Overall, the results indicate that alginate and fucoidan exert distinct effects on bacterial cellulose gel microstructure.

Regarding the chemical composition, the gels contained 34.6% (dry weight) alginate and 28.4% (dry weight) fucoidan. Previous studies on *in situ* production of bacterial cellulose-alginate systems have determined the incorporation of alginate in the BC network using FTIR (Kanjamosit et al., 2010; Zhou et al., 2007) however, the level of incorporation was not quantified making direct comparison difficult. In another study (Gromovkykh et al., 2025), no incorporation of alginate was observed unless calcium was added, which was attributed to the washing steps that likely promoted alginate leaching from the gel network. In contrast, in the present study, a cold alginate solution was used for washing, which likely reduced alginate leaching and prevented its loss from the network. Previous studies on plant cell wall polysaccharides have reported varying levels of incorporation in the

Table 1

Weight-average molecular weight (M_w), number average molecular weight (M_n), polydispersity index (M_w/M_n), ζ potential, ratio of guluronate to mannuronate (G:M) and viscosity of alginate and fucoidan (0.5% w/v in 10 mM NaCl). Average and standard deviation of three replicates ($n = 3$), n.m. = not measured.

	M_w (kDa)	M_n (kDa)	M_w/M_n	ζ potential	G:M	Viscosity at 10 s ⁻¹ (mPa s)
Alginate	343 ± 26	215 ± 19	1.59	-40.5 ± 1.10	0.17	81.6 ± 1
Fucoidan	1038 ± 22	573 ± 38	1.81	-25.2 ± 0.73	n.m.	2.3 ± 0.8

Table 2

Bacterial cellulose gels thickness, wet weight, solid content, density (calculated for wet samples) and chemical composition. Alginate incorporation was determined by total uronics assay. Fucoidan was estimated from fucose content measured by HPLC. ¹Average and standard deviation of 10 replicates ($n = 10$), ²average and standard deviation of 3 replicates ($n = 3$), ³ two analytical measurements ($n = 2$). Different letters within the same row denote significant differences (confidence interval of 95%).

	BC	BC_Alginat	BC_Fucoidan
Thickness (mm) ¹	1.97 ± 0.64 ^a	2.74 ± 0.54 ^a	1.98 ± 0.17 ^a
Wet weight (g) ¹	1.93 ± 0.25 ^a	2.78 ± 0.33 ^b	1.38 ± 0.26 ^c
Dry weight (g) ²	0.022 ± 0.004 ^a	0.040 ± 0.007 ^b	0.024 ± 0.005 ^a
Density (g/cm ³) ¹	0.012 ± 0.004 ^a	0.016 ± 0.003 ^a	0.015 ± 0.004 ^a
Polysaccharide concentration(g/100g) ²	1.14 ± 0.28 ^a	1.42 ± 0.36 ^a	1.74 ± 0.43 ^a
Cellulose (% dry weight) ³	100	65.4	71.6
Alginate incorporation (% dry weight) ³	0	34.6	0
Fucoidan incorporation (%dry weight) ³	0	0	28.4
Cellulose concentration (g/100g) ³	1.1	0.9	1.2
Ratio cellulose/polysaccharide ³	1:0	1:0.5	1:0.4

bacterial cellulose networks depending on the polymer type. For arabinoxylan, incorporation levels are typically in the range of 8–12% (Mikkelsen et al., 2015), although values as high as 52% have been reported (Lopez-Sanchez et al., 2015). In the case of mixed-linkage glucans, incorporation can reach approximately 30% (Mikkelsen et al., 2015). Xyloglucan has been reported to incorporate at levels between 22% and 37% (Chanliaud et al., 2002; Lopez-Sanchez et al., 2015) while pectin showed incorporation ranging from 20 to 60% (Chanliaud et al., 2002; Lopez-Sanchez et al., 2016).

The cellulose concentration in the gels was 1.1% for BC, 0.9% in BC_Alginat and 1.2% in BC_Fucoidan. Zhou et al. (Zhou et al., 2007) reported that the presence of sodium alginate could increase bacterial cellulose yield because it prevented the formation of large cellulose clumps and promoted the dispersion of discrete cellulose masses throughout the culture medium, thereby improving cellulose production efficiency. In this study, BC and BC_Alginat gels exhibited comparable cellulose content; however, differences in cultivation systems and the molecular features of alginate may influence cellulose deposition. To the best of our knowledge, no studies on the *in situ* synthesis of bacterial cellulose–fucoidan gels have yet been reported.

3.3. Microstructure of cellulose gels

In addition to the chemical composition, the impact of the non-cellulosic polysaccharides on the gel structure was investigated by scanning electron microscopy (SEM). Scanning electron micrographs revealed that the BC gels were characterised by a network of randomly oriented cellulose fibres with diameters of 50–100 nm (Fig. 1A), consistent with previous studies (Lopez-Sanchez et al., 2014; Whitney et al., 1995). The presence of alginate during cellulose synthesis led to the formation of gels in which the cellulose fibres remained visible, but were surrounded by a dense background, a smooth surface appears filling up the pores and coating the surface of cellulose ribbons (Fig. 1B and D), this feature may be attributed to alginate. Similar features have

been earlier observed for BC_Alginat films (Kanjamosit et al., 2010). The ratio G:M of the alginate was 0.17, the higher conformational flexibility and more extended structure of M-rich alginate may facilitate its interaction with cellulose through increased surface contact and hydrogen bonding, although the affinity is also expected to depend on the specific G/M sequence distribution. In contrast, BC_Fucoidan gels exhibited a markedly different structure (Fig. 1C and E). The cellulose fibres appeared to be coated, resulting in increased apparent fibre thickness and a more heterogeneous surface morphology. The micrographs suggest that fucoidan may associate with or aggregate on the surface of cellulose fibres, potentially leading to surface deposition and structural heterogeneity within the gel network. However, it is also possible that fucoidan aggregates formed during the sample preparation step involving methanol. The microstructure of BC_Alginat is similar to that reported for BC_Pectin gels (Fanta et al., 2012; Lopez-Sanchez et al., 2016; McKenna et al., 2010), in which pectin appeared also as a smooth dense surface in SEM images. This similarity may be attributed to the comparable chemical features of alginate and pectin, as both are anionic polysaccharides composed of uronic acid residues that confer a high density of carboxyl groups. On the other hand, deposition of polysaccharides on cellulose fibres has been reported for mixed linkage β -glucans (Mikkelsen et al., 2015) and arabinoxylan (Lopez-Sanchez et al., 2014; Mikkelsen et al., 2015).

3.4. Molecular organisation in cellulose gels with alginate and fucoidan

FTIR spectroscopy was used to further investigate the composition and polysaccharide interactions in the gels. FTIR spectra were recorded over the complete spectral range of 4000–400 cm⁻¹. However, only the 1800–800 cm⁻¹ region is presented in Fig. 2A, as it encompasses the characteristic fingerprint bands of polysaccharides (Kačuráková et al., 2002; Wilson et al., 2000) and highlights the most relevant spectral differences among BC, BC_Alginat and BC_Fucoidan. In the fingerprint region, the band at 1632 cm⁻¹ was attributed to the H–O–H bending vibration of absorbed water associated with the hydrophilic cellulose network (δ H–O–H), the band at ~1409 cm⁻¹ is related to CH₂ bending vibrations (δ CH₂), while signals at ~1336 cm⁻¹ arise from bending vibration of the glucopyranose ring (δ CH). Strong absorption bands observed at ~1160, 1109, 1056, and 1030 cm⁻¹ are characteristic of C–O–C glycosidic and glucopyranose ring related C–O/C–C stretching vibrations, with contributions from O–CH bending in cellulose (ν (C–O–C); ν (CO) ν (CC); ν (CO) ν (CC) δ (OCH); ν (CO) ν (CC) ν (CCO)) (Kačuráková et al., 2002; Wilson et al., 2000). The high cellulose content, compared to that of alginate and fucoidan, caused the BC_Alginat and BC_Fucoidan spectra to be dominated by the intense absorption of cellulose bands. Nevertheless, the FTIR spectra confirmed the successful incorporation of alginate and fucoidan into the cellulose gels (Fig. 2A). In the BC_Alginat sample, the band at ~1632 cm⁻¹ observed in BC shifted to lower wavenumbers (~1602 cm⁻¹), reflecting the contribution of the asymmetric stretching vibration of alginate carboxylate groups (ν (COO)) overlapping with the cellulose-associated water band, as previously reported for similar gels (Kanjamosit et al., 2010). Furthermore, bands are observed around ~1418 and ~1315 cm⁻¹, these bands are of slightly higher intensity than in BC. In alginate these bands are attributed to symmetric stretching of carboxylate groups and C–O vibrations, these features may overlap with cellulose signals within the same wavenumber range (Kanjamosit et al., 2010). For the BC_Fucoidan gel, a distinct peak appears at ~1227 cm⁻¹, corresponding to the

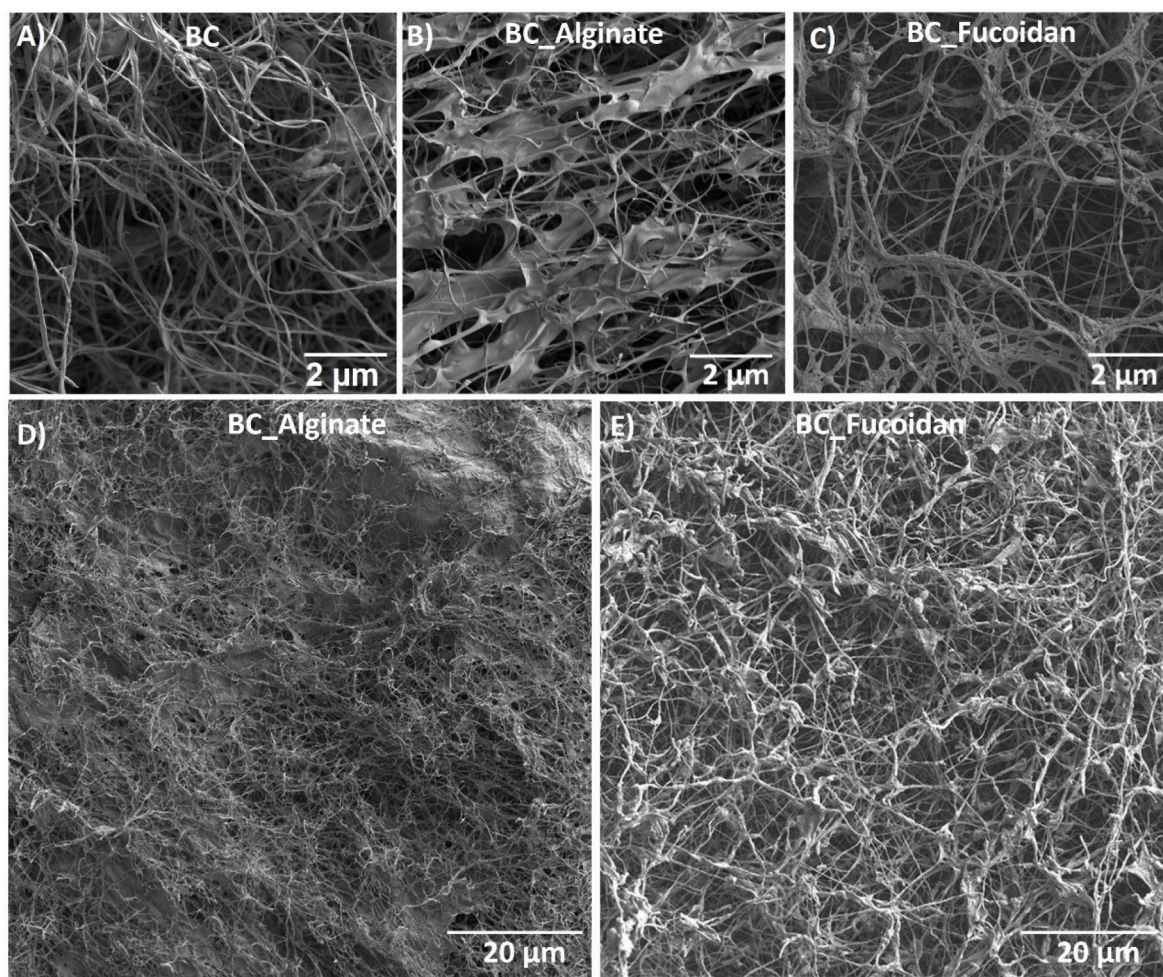


Fig. 1. Scanning electron micrographs of A) bacterial cellulose, B) bacterial cellulose gels produced in the presence of alginate and, C) bacterial cellulose gels produced in the presence of fucoidan. A larger overview of the BC_{Alginate} and BC_{Fucoidan} gels is shown in D) and E).

asymmetric stretching vibration of sulfate ester groups ($\nu(\text{S}=\text{O})$), a characteristic feature of sulphated polysaccharides such as fucoidan (Palanisamy et al., 2017). The signal observed near $\sim 1734\text{ cm}^{-1}$ may be attributed to carbonyl ($\text{C}=\text{O}$) stretching, suggesting the presence of ester or uronic acid groups, which could be related to the fact that the fucoidan contained ca. 5% alginate. In addition, signals $964\text{--}824\text{ cm}^{-1}$ are related to polysaccharide stretching vibrations involving glycosidic linkages and ring deformations and may be associated with structural features of fucoidan (Yang & Zhang, 2009). FTIR indicated that both alginate and fucoidan were incorporated in the cellulose network but there was no evidence for strong chemical interactions.

^{13}C NMR spectroscopy (Fig. 2B) was used to investigate molecular structure and mobility within the gels. Under conditions of cross-polarization combined with magic-angle spinning, signals from rigid regions are observed. By integrating the resonances between 85–92 ppm and 80–86 ppm, which correspond to crystalline and amorphous C4 carbon sites in cellulose, an overall crystallinity of approximately 82% was determined for the BC gels (Table 3). Analysis of this spectral region also enabled estimation of the polymorphic composition, giving an α : β ratio of 56:44. This is consistent with reported values for bacterial cellulose, in which the α form is predominant (Lopez-Sanchez et al., 2015). For BC_{Alginate} and BC_{Fucoidan} the values of crystallinity were slightly lower 73% and 75% respectively. There were no signals for either alginate or fucoidan in CP/MAS spectra (Fig. 2B), suggesting that these polymers are not in a rigid/solid phase in the gels. Thus, there was no evidence of molecular associations between cellulose and alginate or

fucoidan, either at the point of cellulose chain aggregation, which would lead to changes in crystallinity, or subsequently, resulting in immobilisation of alginate or fucoidan. Previous studies on hemicelluloses (Mikkelsen et al., 2015) and pectins (Lopez-Sanchez et al., 2016) have been reported to have negligible impact on the crystalline structure of cellulose. In contrast, xyloglucan (Lopez-Sanchez et al., 2015; Whitney et al., 1995) have been shown to interact strongly with cellulose microfibrils, leading to a reduction in crystallinity. In addition, certain mannans (Whitney et al., 1998) have also been reported to interact with bacterial cellulose and reduce its crystallinity. Microscopic observations and their interpretation should be approached with caution. However, when considered together with the chemical results obtained from FTIR and NMR analyses, the findings strongly suggest that no new chemical bonds are formed between alginate or fucoidan and cellulose. Instead, the interaction appears to be predominantly physical, most likely involving adsorption onto the surface of the cellulosic fibres.

Based on previous studies (Lopez-Sanchez et al., 2016) cellulose diffraction patterns (Fig. 2C) were considered to arise from three reflections, at 14.3° , 16.9° and 22.8° (corresponding to the cellulose α allomorph 100, 010 and 110 crystal planes respectively), the amorphous halo could be centred at approximately 18° . The crystallinity index calculated from XRD data (Table 3) is in good agreement with the values obtained from NMR analysis and indicates that the presence of either alginate or fucoidan does not significantly affect the crystallinity of cellulose. Regarding crystallite dimensions, only minor differences were observed. In the case of fucoidan, an increase in $D_{(100)}$ was noted,

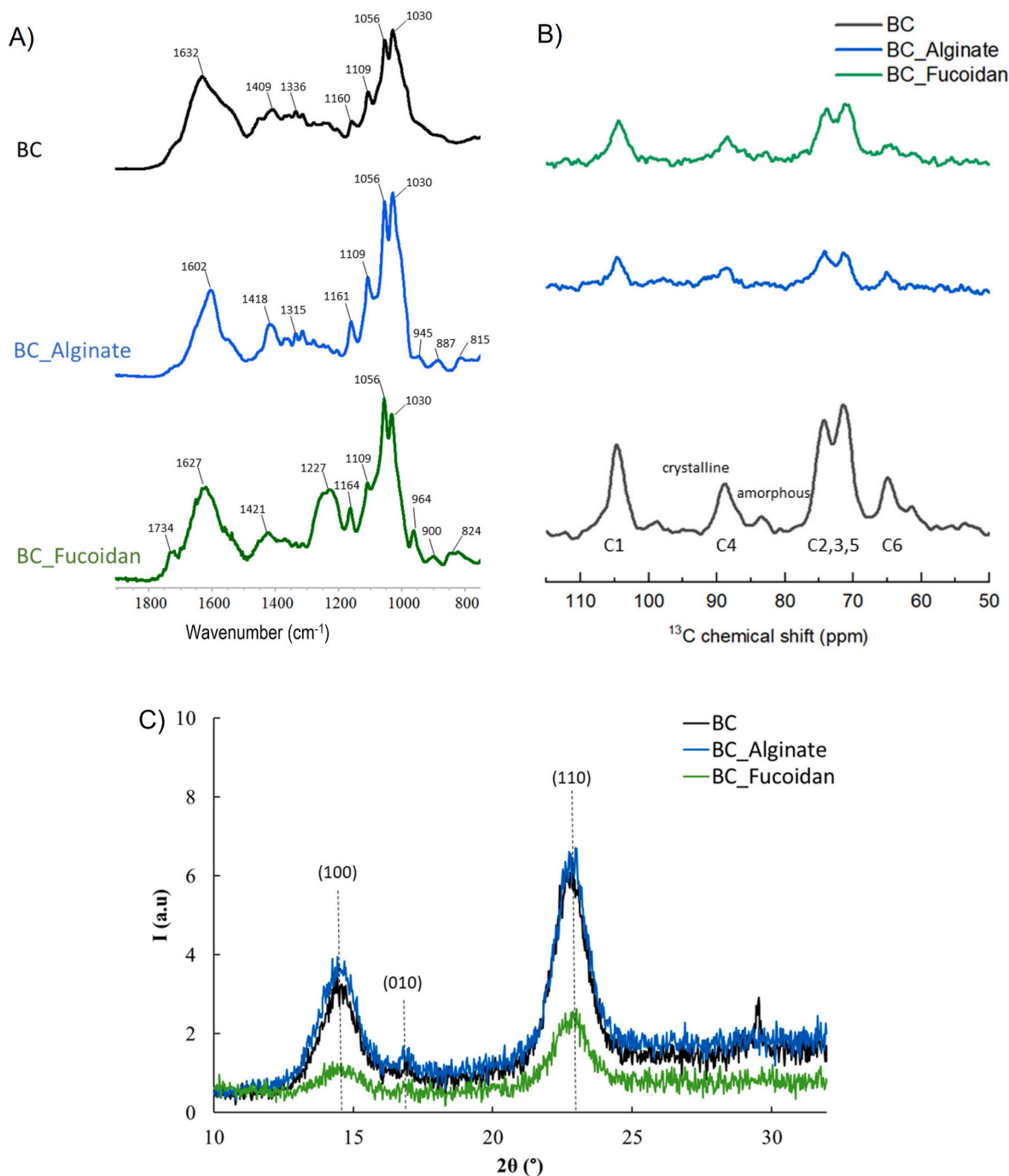


Fig. 2. A) FTIR spectra of dried cellulose gels (BC), cellulose/alginate (BC_Alginat) gels and cellulose/fucoidan (BC_Fucoidan) gels. B) ¹³C CP/MAS spectra of wet samples. C) XRD patterns of dried cellulose gels (BC), cellulose/alginate (BC_Alginat) gels and cellulose/fucoidan (BC_Fucoidan) gels.

whereas alginate appeared to increase D₍₁₁₀₎. However, given the small magnitude of these differences, and the absence of changes in lattice spacing, it remains unclear whether they reflect genuine structural modifications or fall within the experimental uncertainty of the measurements. The absence of any detectable shift in d-spacing for both alginate and fucoidan containing gels suggests that the native cellulose I crystal lattice remains unaffected, confirming that neither polysaccharide penetrates the crystalline domains. Instead, both polymers are likely to interact primarily at the surface of cellulose fibrils and within amorphous regions.

3.5. Impact of alginate or fucoidan on the rheological properties of bacterial cellulose gels

The role of alginate and fucoidan on the rheological properties of the gels was studied by small amplitude oscillatory shear. All samples showed a storage modulus G' larger than the loss modulus G'' , consistent with gel-like behaviour (Table 4). Gel strength, indicated by G' , was 1 order of magnitude higher for BC than the for the other gels (4677 ± 620 Pa). BC_Fucoidan showed intermediate G' values of (598 ± 97 Pa) whilst BC_Alginat was the softest, with G' values of 302 ± 98 Pa. The elasticity in bacterial cellulose gels has been proposed to arise mainly from the effective number of physical entanglements

Table 3

Crystallinity index (X_c), cross-sectional dimensions of crystallite sizes in the direction perpendicular to the (100), (010) and (110) planes (D_{100} , D_{010} , D_{110}) and corresponding d-spacings, determined from the XRD patterns of the dried bacterial cellulose (BC), bacterial cellulose with alginate (BC_{Alginate}) and bacterial cellulose with fucoidan (BC_{Fucoidan}). D_{010} was not determined (n.d) due to low signal/noise. Crystallinity values calculated from NMR signals are also included.

	BC	BC _{Alginate}	BC _{Fucoidan}
X_c (%) XRD	87.8	84.8	83.9
X_c (%) NMR	82	73	75
D_{100} (nm)	5.3	5.2	6.1
D_{010} (nm)	n.d	n.d	n.d
D_{110} (nm)	5.5	6.1	5.5
d_{100} (Å)	6.2	6.2	6.2
d_{010} (Å)	n.d	n.d	n.d
d_{110} (Å)	3.8	3.8	3.8

Table 4

Storage and loss moduli, compression stress and Young's modulus of bacterial cellulose gels (BC), bacterial cellulose with alginate (BC_{Alginate}) and bacterial cellulose with fucoidan (BC_{Fucoidan}). Data are presented as mean $\pm$ SD of three replicates ($n = 3$). Different letters within the same row denote significant differences (confidence interval of 95%).

	BC	BC _{Alginate}	BC _{Fucoidan}
Storage modulus G' (Pa)	4677 $\pm$ 620 ^a	302 $\pm$ 98 ^c	598 $\pm$ 97 ^b
Loss modulus G'' (Pa)	2490 $\pm$ 287 ^a	137 $\pm$ 38 ^c	262 $\pm$ 8.1 ^b
Compression stress at 10% strain (Pa)	2323 $\pm$ 402 ^a	419 $\pm$ 143 ^c	1476 $\pm$ 218 ^b
Young's modulus (kPa)	22.6 $\pm$ 6.2 ^a	12.8 $\pm$ 2.8 ^b	15.8 $\pm$ 0.4 ^b

between cellulose fibrils, observed in scanning electron images (Lopez-Sanchez et al., 2014; Whitney et al., 1999). The fact that G' decreases when alginate or fucoidan is present during cellulose synthesis indicates that either the gels contain less cellulose and/or that the number of fibril entanglements per unit volume is lower. According to the gel composition (Table 2), the cellulose concentration among the gels was rather similar, 0.9–1.2%; thus, it is likely that the lower G' in the BC_{Alginate} is due to a reduced number of entanglements compared to BC. The viscosity of the medium in which the cellulose is produced could impact the effective number of entanglements, as previously shown for gels produced in the presence of pectin of increasing concentrations (Lopez-Sanchez et al., 2016). In this case, the viscosity of the alginate solution is approximately 80 times higher than that of water (Table 1). In contrast, the viscosity of the fucoidan solution (2 mPa s) is close to water viscosity. Therefore, if there is a reduced number of contact points between cellulose fibres in the BC_{Fucoidan}, this is unlikely to be due to viscosity effects during cellulose deposition, but rather to other factors.

Fig. 3A shows the evolution of G' after a series of compression cycles. Previous studies have shown that, during compression, the fluid phase is squeezed out of this type of gels (Lopez-Sanchez et al., 2014). Owing to their near-zero Poisson's ratio, the materials do not expand in the radial direction; instead, they collapse in the direction of compression, promoting the formation of new cellulosic entanglements. All three gel types showed an increase in G' after compression, suggesting that, as the samples are compressed, the effective number of entanglements per unit volume increases, either because the cellulose fibres move closer together or because they become reoriented during compression, leading to an increase in G' . Fig. 3B shows that, when normalised to the initial modulus ($G'(t_0)$), the BC_{Alginate} gels exhibited the largest relative increase in G' during compression. However, the absolute increase in G' was substantially lower than that observed for pure BC (Fig. 3C). This apparent discrepancy arises from the much lower initial modulus of the BC_{Alginate} samples, meaning that even a modest absolute increase in G' corresponds to a large relative change after normalisation. These results suggest that the BC_{Alginate} gels undergo a greater degree of

compression-induced structural reorganisation relative to their initial state, rather than forming a larger total number of load-bearing entanglements than pure BC. In pure BC gels, cellulose ribbons are already in close proximity prior to compression, as no other polysaccharides are present between the fibres or coat their surfaces, as observed in the SEM images (Fig. 1). This results in a mechanically stronger and more interconnected network. Consequently, compression leads to a large absolute increase in G' , likely due to the formation of additional effective cellulose–cellulose contacts and entanglements. In contrast, the presence of alginate appears to initially reduce the effectiveness of cellulose–cellulose interactions, producing a weaker and more open network structure. During compression, water is expelled from the gel and, owing to its high hydrophilicity and weak attachment to the cellulose network, alginate may also be partially squeezed out together with the aqueous phase. This process would progressively increase the local cellulose concentration and promote closer packing and reorientation of cellulose ribbons, leading to a proportionally large increase in network connectivity and stiffness. Nevertheless, despite this pronounced relative reinforcement, the final compressed BC_{Alginate} networks remain mechanically weaker than pure BC, as reflected by their lower absolute G' values. The BC_{Fucoidan} gels displayed an intermediate behaviour between pure BC and BC_{Alginate} systems (Fig. 3A). As shown in Fig. 3C, the absolute increase in G' after compression was similar to that observed for BC_{Alginate}, and substantially lower than for pure BC. In the normalised representation (Fig. 3B), the relative increase in G' was moderate and remained closer to that of pure BC than to the BC_{Alginate} gels. This suggests that, although compression promotes additional network formation in the presence of fucoidan, the extent of structural rearrangement is less pronounced than in the BC_{Alginate} gels.

The SEM observations provide important insight into the rheological behaviour. Unlike alginate, which is likely distributed within the aqueous phase and may be partially expelled during compression together with water, fucoidan appeared deposited or coated directly onto the cellulose ribbons. This morphology suggests stronger or more persistent associations between fucoidan and the cellulose network. Such coating of the cellulose fibres may partially hinder direct cellulose–cellulose contact formation during compression, limiting the magnitude of the absolute increase in G' relative to BC. At the same time, because fucoidan remains associated with the fibrillar network rather than being readily expelled, the initial network is likely more structurally integrated and less prone to large-scale rearrangement during compression than the BC_{Alginate} gel.

3.6. Compression-relaxation behaviour of bacterial cellulose gels with alginate or fucoidan

The mechanical properties of the gels were evaluated by compression-relaxation experiments. Regarding the resistance to compression of the gels, the maximum compressive stress at 10% strain was 2323 Pa for BC, 419 Pa for BC_{Alginate}, and 1476 Pa for BC_{Fucoidan} (Table 4). Similarly, the Young's modulus decreased from 22.6 kPa for BC to 12.8 kPa for BC_{Alginate}, while BC_{Fucoidan} exhibited an intermediate value of 15.8 kPa. Since no evidence of specific interactions between alginate and bacterial cellulose was observed, the lower compressive strength and stiffness of the BC_{Alginate} gels is likely due to a lower cellulose concentration and reduced effective density of load-bearing cellulose fibril contacts. The viscosity of alginate (Table 1) appears insufficient to contribute to compressive resistance via poroelastic effects, as previously reported for pectin gels (Lopez-Sanchez et al., 2016). Fig. 4 shows the compression-relaxation behaviour of the gels during successive compression cycles. Native bacterial cellulose (BC) exhibited elastic behaviour during the first compression cycle (Fig. 4A), as evidenced by the complete stress relaxation upon unloading (Fig. 4B). The relaxation behaviour typically consists of a rapid decrease in stress. However, with increasing deformation and repeated compression cycles, BC displayed progressively more viscoelastic

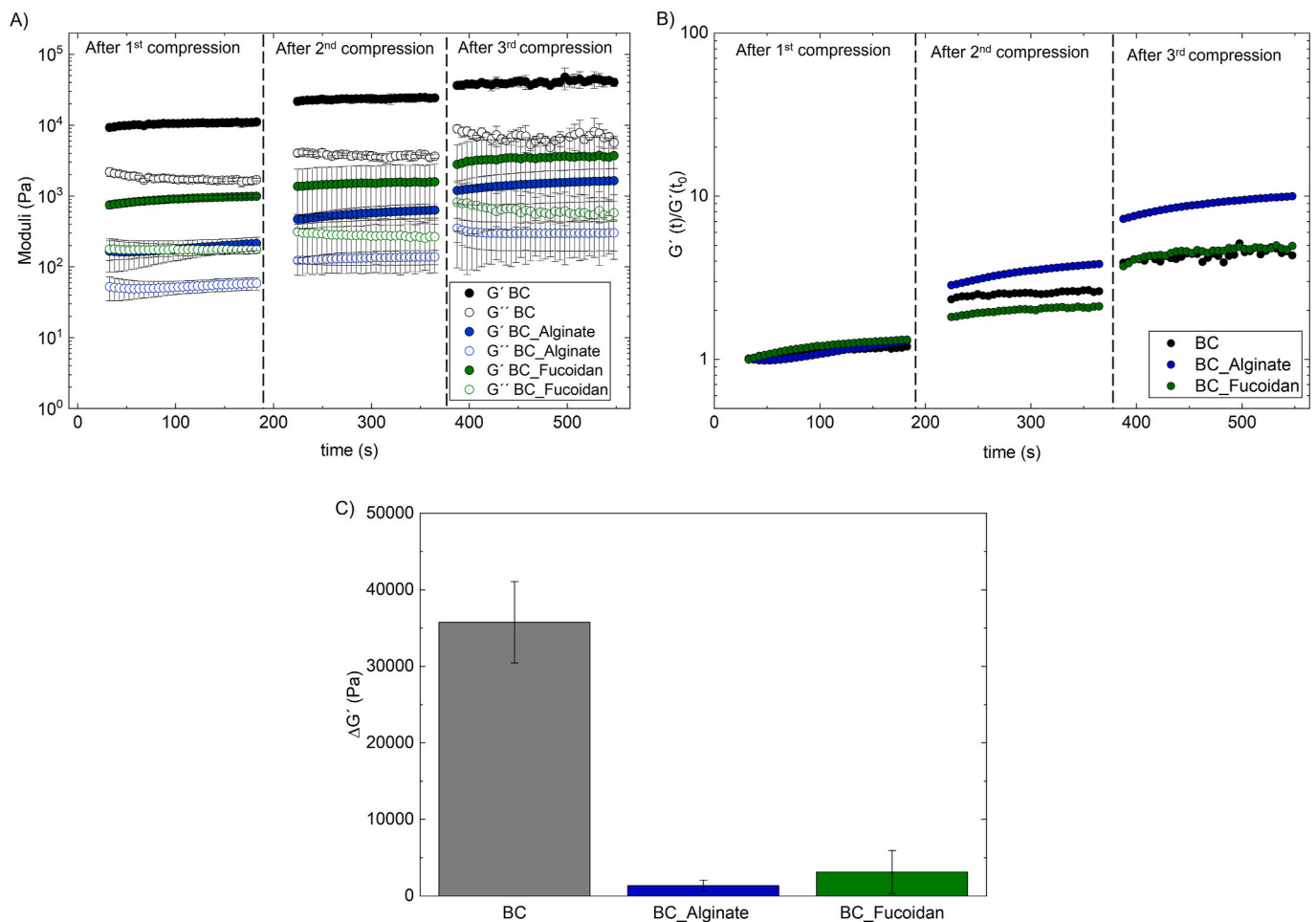


Fig. 3. A) Storage modulus (G') and loss modulus (G'') after compression of bacterial cellulose gels. B) Normalised storage modulus of the same samples. C) Overall increase in G' after compression for the gels BC, BC_Alginate and BC_Fucoidan. Data are presented as average $\pm$ SD of three replicates ($n = 3$).

behaviour (Fig. 4C–F), characterised by incomplete stress recovery after unloading. Residual stresses of 320 Pa and 886 Pa were measured after the second and third compression cycles, respectively (Table 5), indicating increasing structural rearrangement and energy dissipation within the cellulose network at higher strains. Cellulosic gels have been reported to undergo complete or near-complete stress recovery during the relaxation step, indicating an ability to accommodate structural changes induced by deformation (Lopez-Sanchez et al., 2014). The effect of compression rate on cellulose networks has been demonstrated previously, with fast compression speeds, similar to the ones in our study, leading to faster relaxation. In contrast, alginate containing gels maintained predominantly elastic behaviour throughout all compression cycles, even at the highest deformation levels investigated (Fig. 4A–C and E). BC_Alginate gels and BC_Fucoidan exhibited complete stress relaxation after unloading, including during the third compression cycle (Fig. 4F), suggesting that the imposed deformation remained largely reversible. Previous studies on BC_Pectin gels (Lopez-Sanchez et al., 2016) compressed at lower rates (1 $\mu\text{m/s}$) than those employed in this study (100 $\mu\text{m/s}$) have shown viscoelastic behaviour under compression, characterised by an initial linear elastic regime followed by plastic deformation. The stress relaxation response typically consisted of a rapid decrease in stress followed by complete relaxation, similar to the behaviour observed for BC_Alginate gels.

The distinct mechanical responses of BC and BC_Alginate gels at high deformations are likely related to differences in network architecture and deformation mechanisms. In BC gels, compression at larger strains promotes irreversible rearrangement of cellulose fibrils, disruption of

inter-fibrillar hydrogen bonding, and localised network densification. These processes dissipate mechanical energy and generate a time-dependent viscoelastic response, resulting in incomplete recovery after unloading. In contrast, the BC_Alginate network contains a larger mobile aqueous phase that can redistribute within the porous structure during compression. This fluid mediated deformation reduces stress concentrations within the cellulose network and limits irreversible fibrillar rearrangements. As a result, BC_Alginate gels retain a predominantly elastic response and exhibit complete recovery even after repeated compression cycles.

These findings suggest that the viscoelasticity observed in BC at high strains arises primarily from structural reorganisation of the cellulose network, whereas the presence of alginate solution within the pore space promotes reversible deformation by facilitating stress redistribution without contributing significantly to network reinforcement.

Brown algal cell walls are complex, heterogeneous, three-dimensional structures composed of a variety of polysaccharides, including alginate, fucoidan, and cellulose, as well as structural proteins and phenolic compound. In addition, the relative abundance, distribution, and organisation of these components can vary according to species, tissue type, developmental stage, and environmental conditions. The bacterial cellulose model used here necessarily simplifies this complexity by focusing primarily on polysaccharide-polysaccharide interactions within a controlled cellulose network. Therefore, it does not reproduce the complete chemical composition, spatial organisation, or biological complexity of a native brown algal cell wall. In particular, potential contributions from proteins, phenolic compounds, ionic cross-

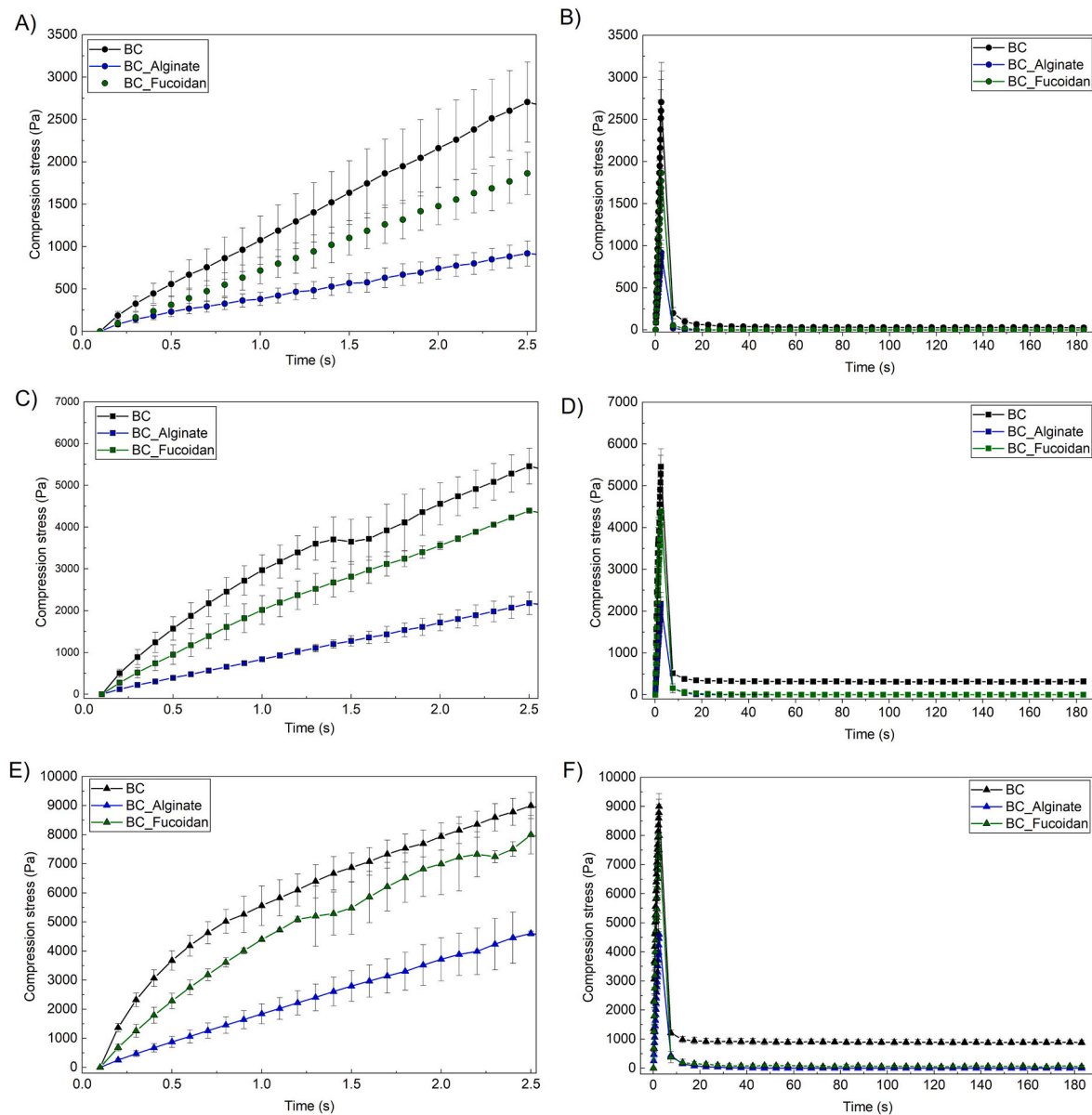


Fig. 4. Compression and stress relaxation curves for cycle 1 (A, B) cycle 2 (C,D) and cycle 3 (E,F) corresponding to increasing strains (due to the differences in initial thickness for cycle 1 BC and BC_Fucoidan reached 12% strain whilst BC_Algininate reached 7.5%; for cycle 2: BC and BC_Fucoidan reached 25% and alginate 15%; for cycle 3: BC and BC_Fucoidan reached 37% and BC_Algininate 23%). Curves represent average and standard deviation of three measurements ($n = 3$).

Table 5

Residual stress after different cycles of compression at 100 $\mu\text{m/s}$ corresponding to increasing strains. Data are presented as mean $\pm$ SD of three replicates ($n = 3$).

	Residual stress after Cycle 1 (Pa)	Residual stress after Cycle 2 (Pa)	Residual stress after Cycle 3 (Pa)
BC	25.7 ± 22.9	319.7 ± 19.09	885.7 ± 84.2
BC_Algininate	0	0	0
BC_Fucoidan	0	0	55.1 ± 46.8

linking, and other cell-wall components are not represented. Nevertheless, the mechanical behaviour observed for BC_Algininate gels may provide insights into the structural role of alginate in brown seaweed cell walls. While our model system does not reproduce the full compositional complexity of native algal cell walls, it is a first approach to capture key aspects of the physical interactions between cellulose and alginate. In our gels, the cellulose microfibril network provides tensile reinforcement, whereas the alginate matrix contributes to hydration, flexibility,

and reversible deformation under mechanical loading, suggesting that alginate may act as a stress-distributing phase, preventing irreversible rearrangement of the cellulose framework. Such a mechanism would allow seaweed tissues to withstand repeated hydrodynamic forces generated by waves and currents while maintaining structural integrity and flexibility.

3.7. Processing of bacterial cellulose gels with alginate and fucoidan: impact of thermal treatment, sonication and rehydration

The selected treatments were intended to represent commonly used processing conditions that can substantially modify the structure, hydration state, and extractability of cell-wall polysaccharides, thereby impacting cell wall structure. Thermal processing is commonly applied to algae and can promote polysaccharide solubilisation and changes in network structure. Ultrasound was selected as a physical treatment capable of modifying hydrated polysaccharide networks and potentially promoting the release or redistribution of polymeric components.

Lyophilisation followed by rehydration was included to examine the effects of dehydration and subsequent rehydration on the organisation and integrity of the polysaccharide network. These treatments allowed us to investigate whether changes in network structure could alter the rheological properties of the gels. Such information may help to interpret how processing-induced structural changes could contribute to changes in algal cell wall properties. As shown in Fig. 5A, the weight of BC and BC_Fucoidan gels remained unchanged after thermal and ultrasound treatment, indicating that no significant solubilisation or loss of polysaccharide occurred under the applied conditions. In the case of BC, this behaviour was expected due to the thermal stability of bacterial cellulose within the temperature range employed in this study. For BC_Fucoidan gels, these results suggest that fucoidan remained strongly associated with the cellulose network, and that washing in hot water at 90 °C for 30 min was insufficient to disrupt these interactions or remove

the polysaccharide from the matrix. Among other methods, fucoidan can be extracted from brown algae by hot water extraction (60–90 °C) (Palanisamy et al., 2017; Yuk Ko et al., 2026), although usually low yields are obtained with this extraction methods. It is worth noting that the colour of the fucoidan powder was brownish and the gels remain brownish after washing. As shown by SEM (Fig. 1), fucoidan appeared coating the cellulose fibres, since no evidence for chemical interactions was observed this indicates that the fucoidan is physically entrapped in the gel network. In contrast, BC_Alginates gels exhibited a reduction in weight (Fig. 5A) following thermal treatment, indicating alginate solubilisation from the gel network. This behaviour was also reflected in the rheological properties of the thermally treated gels (Fig. 5B). The removal of alginate during thermal treatment promoted a greater degree of cellulose entanglement and fibrillar interactions during compression of the thermally treated samples, resulting in an increase in G' in

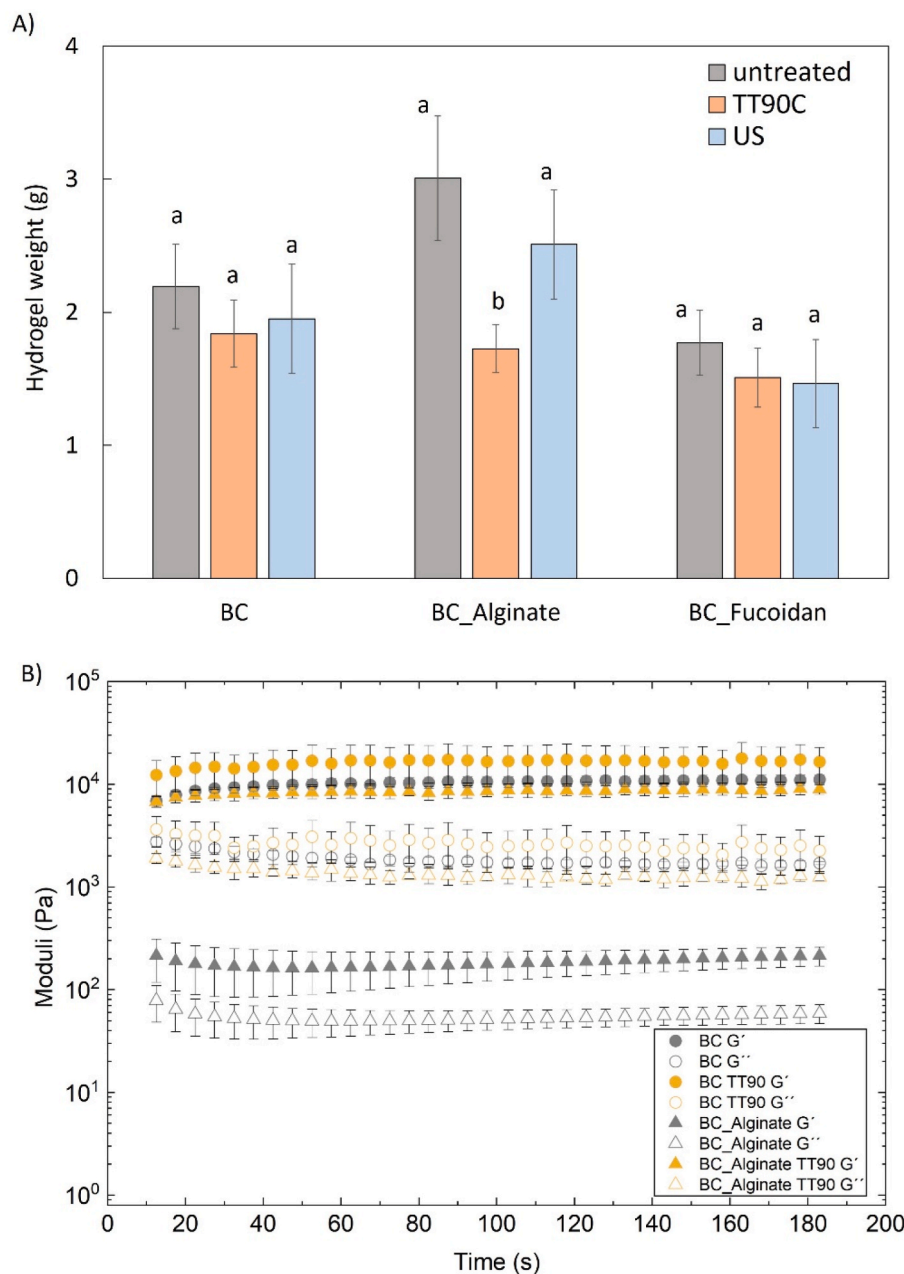


Fig. 5. A) Bacterial cellulose gels weight before and after thermal treatment (90 °C 30 min) and after ultrasounds treatment. Data are presented as mean $\pm$ SD of ten replicates ($n = 10$). Different letters denote significant differences (confidence interval of 95%). B) Moduli after the first compression for the untreated and the thermally treated gels. Data are presented as mean $\pm$ SD of three replicates ($n = 3$).

comparison with untreated samples. The results support our hypothesis that alginate has a load bearing role in the system, reducing collapse of the cellulose network.

None of the gels recovered their original weight after rehydration (Fig. 6), indicating that the structural collapse occurring during freeze-drying was irreversible. This loss of structural integrity likely reduced the water-holding capacity of the gels and impaired their ability to fully reabsorb water upon rehydration. Although there were no significant differences due to the large variability between replicates, characteristic of biological samples, BC_Alginat e gels had a higher recovery than BC and BC_Fucoidan.

4. Conclusions

Overall, the results support that alginate and fucoidan interact with the BC network through distinct physical mechanism. Alginate shows only weak interaction with the cellulose fibre network and, can be removed by hot water washing. It therefore acts primarily as a highly hydrated filling phase within the BC matrix, increasing water retention while simultaneously reducing the effective fibre crosslink density, resulting in gels with lower G' than pure BC. In addition, BC_Alginat e gels showed enhanced mechanical resilience, recovering fully after compressive deformation, in contrast to cellulose-only systems that exhibited irreversible structural changes.

Thus, alginate appears to influence the BC network mainly through its hydration and space-filling effects rather than through strong association with the cellulose fibres.

In contrast, fucoidan shows a stronger affinity for cellulose fibrils, remaining associated with the material after washing. Microscopic and structural evidence suggests that fucoidan is likely adsorbed onto the cellulose surface, forming a coating layer, however, this interaction is physical, with no evidence of the formation of covalent bonds. In addition, cellulose crystallinity was not influenced by the presence of fucoidan, indicating that cellulose assembly is not disrupted at the molecular level. BC_Fucoidan gels also showed lower G' than pure BC, indicating interference with BC network formation, likely by reducing the effective number of cellulose fibril entanglements. Thus, unlike alginate, fucoidan appears to modify the BC network more directly through its association with the cellulose fibre surface.

The different behaviour of alginate and fucoidan highlights the importance of polysaccharide characteristics in determining their interactions with the BC network. Molecular weight and ζ -potential may influence the behaviour of alginate and fucoidan within the BC network by affecting polymer mobility, chain entanglement, and electrostatic interactions. However, they should be considered alongside other factors, such as polysaccharide composition (G:M ratio), degree of sulphation, and the structural characteristics of the cellulose network. Taken together, these findings advance the understanding of how structurally different algal polysaccharides differentially regulate the structural, mechanical and rheological behaviour of bacterial cellulose gels, providing a biomimetic framework for interpreting their potential roles in native algal cell wall architecture.

CRedit authorship contribution statement

Patricia Cazón: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Marta Area:** Writing – original draft, Investigation, Formal analysis. **Andrea Crespo:** Writing – review & editing, Investigation, Formal analysis. **Jesus Valcarcel:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Maria Teresa Ahumada Ochoa:** Investigation, Formal analysis. **Mathias Johansson:** Writing – review & editing, Investigation, Formal analysis. **Jummi Laishram:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Regina Ciano:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis. **Anna Ström:** Writing – review & editing, Supervision,

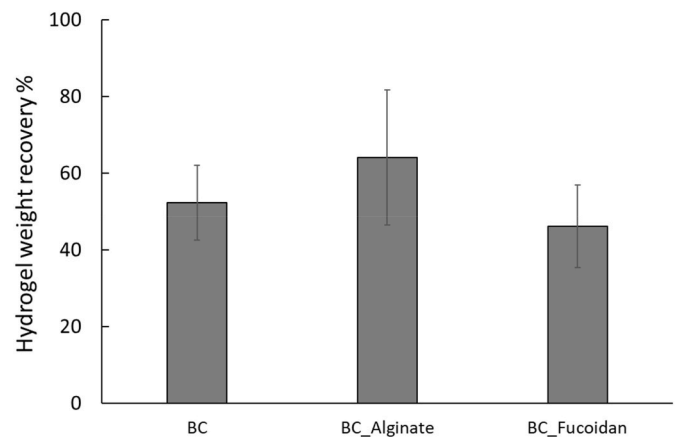


Fig. 6. Bacterial cellulose gels weight recovery after rehydration. Data are presented as mean $\pm$ SD of ten replicates ($n = 10$).

Methodology. **Patricia Lopez-Sanchez:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, 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.

Acknowledgments

The research leading to these results was supported by project PCI2024-153421/PMICIU/AEI/10.13039/501100011033/Union European and, PID2022-138328OB-C22 (Ministerio de Ciencia e Innovacion, Spain). Authors acknowledge ReMade project and CERIC-ERIC Consortium for the access to experimental facilities and financial support (project no. 38258). Funded by the European Union as part of the Horizon Europe call HORIZON-INFRA-2021-SERV-01 under grant agreement number 101058414 and co-funded by UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee (grant number 10039728) and by the Swiss State Secretariat for Education, Research and Innovation (SERI) under contract number 22.00187. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the UK Science and Technology Facilities Council or the Swiss State Secretariat for Education, Research and Innovation (SERI). Neither the European Union nor the granting authorities can be held responsible for them. We also acknowledge the use of the XRD infrastructure at Chalmers Materials Analysis Laboratory (CMAL).

Data availability

Data will be made available on request.

References

- Baghel, R. S., Reddy, C. R. K., & Singh, R. P. (2021). Seaweed-based cellulose: Applications, and future perspectives. *Carbohydrate Polymers*, 267, Article 118241. <https://doi.org/10.1016/j.carbpol.2021.118241>
- Bilan, M. I., Grachev, A. A., Shashkov, A. S., Kelly, M., Sanderson, C. J., Nifantiev, N. E., & Usov, A. I. (2010). Further studies on the composition and structure of a fucoidan preparation from the brown alga *Saccharina latissima*. *Carbohydrate Research*, 345 (14), 2038–2047. <https://doi.org/10.1016/j.carres.2010.07.009>
- Chanliaud, E., Burrows, K., Jeronimidis, G., & Gidley, M. (2002). Mechanical properties of primary plant cell wall analogues. *Planta*, 215(6), 989–996. <https://doi.org/10.1007/s00425-002-0783-8>
- Deniaud-Bouët, E., Kervarec, N., Michel, G., Tonon, T., Kloareg, B., & Hervé, C. (2014). Chemical and enzymatic fractionation of cell walls from fucals: Insights into the

- structure of the extracellular matrix of brown algae. *Annals of Botany*, 114(6), 1203–1216. <https://doi.org/10.1093/aob/mcu096>
- Draget, K. I., Skjakbraek, G., Christensen, B. E., Gaserod, O., & Smidsrod, O. (1996). Swelling and partial solubilization of alginic acid gel beads in acidic buffer. *Carbohydrate Polymers*, 29(3), 209–215. [https://doi.org/10.1016/0144-8617\(96\)00029-X](https://doi.org/10.1016/0144-8617(96)00029-X)
- Draget, K. I., & Taylor, C. (2011). Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocolloids*, 25(2), 251–256. <https://doi.org/10.1016/j.foodhyd.2009.10.007>
- Fanta, S. W., Vanderlinden, W., Abera, M. K., Verboven, P., Karki, R., Ho, Q. T., De Feyter, S., Carmeliet, J., & Nicolai, B. M. (2012). Water transport properties of artificial cell walls. *Journal of Food Engineering*, 108(3), 393–402. <https://doi.org/10.1016/j.jfoodeng.2011.09.010>
- Filiseti-Cozzi, T. M., & Carpita, N. C. (1991). Measurement of uronic-acids without interference from neutral sugars. *Analytical Biochemistry*, 197(1), 157–162. [https://doi.org/10.1016/0003-2697\(91\)90372-Z](https://doi.org/10.1016/0003-2697(91)90372-Z)
- Grasdalen, H., Larsen, B., & Smidsrod, O. (1981). C-13-NMR studies of monomeric composition and sequence in alginate. *Carbohydrate Research*, 89(2), 179–191. [https://doi.org/10.1016/S0008-6215\(00\)85243-X](https://doi.org/10.1016/S0008-6215(00)85243-X)
- Gromovkyh, T. I., Tarasova, I. A., Zefirov, V. V., Gulín, A. A., Ivanenko, I. P., Molchanov, V. S., Kharitonova, E. P., & Kiselyova, O. I. (2025). Composites of bacterial cellulose and alginate produced in situ: The impact of viscosity and temperature on the microscale morphology. *Carbohydrate Polymers*, 357. <https://doi.org/10.1016/j.carbpol.2025.123495>
- Kačuráková, M., Smith, A. C., Gidley, M. J., & Wilson, R. H. (2002). Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D FT-IR spectroscopy. *Carbohydrate Research*, 337(12), 1145–1153. [https://doi.org/10.1016/S0008-6215\(02\)00102-7](https://doi.org/10.1016/S0008-6215(02)00102-7)
- Kanjanamont, N., Muangnapoh, C., & Phisalaphong, M. (2010). Biosynthesis and characterization of bacteria cellulose–alginate film. *Journal of Applied Polymer Science*, 115(3), 1581–1588. <https://doi.org/10.1002/app.31138>
- Kopplin, G., Rokstad, A. M., Melida, H., Bulone, V., Skjak-Braek, G., & Aachmann, F. L. (2018). Structural characterization of fucoidan from laminaria hyperborea: Assessment of coagulation and inflammatory properties and their structure-function relationship. *ACS Applied Bio Materials*, 1(6), 1880–1892. <https://doi.org/10.1021/acsabm.8b00436>
- Kraithong, S., Bunyameen, N., Jaisan, C., Liu, Y. H., Sangsawad, P., Huang, R. M., & Shi, X. (2025). Gelling, thickening, and biological properties of marine algal polysaccharides: Implications for food applications. *Carbohydrate Polymers*, 369. <https://doi.org/10.1016/j.carbpol.2025.124309>
- Lopez-Sanchez, P., Cersosimo, J., Wang, D., Flanagan, B., Stokes, J. R., & Gidley, M. J. (2015). Poroelastic mechanical effects of hemicelluloses on cellulosic hydrogels under compression. *PLoS One*, 10(3). <https://doi.org/10.1371/journal.pone.0122132>
- Lopez-Sanchez, P., Martinez-Sanz, M., Bonilla, M. R., Wang, D., Walsh, C. T., Gilbert, E. P., Stokes, J. R., & Gidley, M. J. (2016). Pectin impacts cellulose fibre architecture and hydrogel mechanics in the absence of calcium. *Carbohydrate Polymers*, 153, 236–245. <https://doi.org/10.1016/j.carbpol.2016.07.113>
- Lopez-Sanchez, P., Rincon, M., Wang, D., Brulhart, S., Stokes, J. R., & Gidley, M. J. (2014). Micromechanics and poroelasticity of hydrated cellulose networks. *Biomacromolecules*, 15(6), 2274–2284. <https://doi.org/10.1021/bm500405h>
- Mazéas, L., Yonamine, R., Barbeyron, T., Henrissat, B., Drula, E., Terrapon, N., Nagasato, C., & Hervé, C. (2023). Assembly and synthesis of the extracellular matrix in brown algae. *Seminars in Cell & Developmental Biology*, 134, 112–124. <https://doi.org/10.1016/j.semcdb.2022.03.005>
- McKenna, B. A., Kopittke, P. M., Wehr, J. B., Blamey, F. P. C., & Menzies, N. W. (2010). Metal ion effects on hydraulic conductivity of bacterial cellulose-pectin composites used as plant cell wall analogs. *Physiologia Plantarum*, 138(2), 205–214. <https://doi.org/10.1111/j.1399-3054.2009.01306.x>
- Mikkelsen, D., Flanagan, B. M., Wilson, S. M., Bacic, A., & Gidley, M. J. (2015). Interactions of arabinoxylan and (1,3)(1,4)- β -Glucan with cellulose networks. *Biomacromolecules*, 16(4), 1232–1239. <https://doi.org/10.1021/acs.biomac.5b00009>
- Mikkelsen, D., Lopez-Sanchez, P., Wang, D., & Gidley, M. J. (2020). Formation of cellulose-based composites with hemicelluloses and pectins using komagataeibacter fermentation. In Z. A. Popper (Ed.), *Plant cell wall* (2 Edition., 2149 pp. 73–87). Methods and Protocols. https://doi.org/10.1007/978-1-0716-0621-6_5
- Morimoto, M., Takatori, M., Hayashi, T., Mori, D., Takashima, O., Yoshida, S., Sato, K., Kawamoto, H., Tamura, J., Izawa, H., Ifuku, S., & Saimoto, H. (2014). Depolymerization of sulfated polysaccharides under hydrothermal conditions. *Carbohydrate Research*, 384, 56–60. <https://doi.org/10.1016/j.carres.2013.11.017>
- Palanisamy, S., Vinosha, M., Marudhupandi, T., Rajasekar, P., & Prabhu, N. M. (2017). Isolation of fucoidan from Sargassum polycystum brown algae: Structural characterization, in vitro antioxidant and anticancer activity. *International Journal of Biological Macromolecules*, 102, 405–412. <https://doi.org/10.1016/j.ijbiomac.2017.03.182>
- Perez-Roa, M. E., Gonzaga, L. J., Lavecchia, R., & Zuurro, A. (2025). Seaweed-type-based protein extraction: A comparative review of green, red, and brown algae. *Algal Research-Biomass Biofuels and Bioproducts*, 91. <https://doi.org/10.1016/j.algal.2025.104221>
- Salomonsen, T., Jensen, H. M., Larsen, F. H., Steuarnagel, S., & Engelsen, S. B. (2009). Alginate monomer composition studied by solution- and solid-state NMR - A comparative chemometric study. *Food Hydrocolloids*, 23(6), 1579–1586. <https://doi.org/10.1016/j.foodhyd.2008.11.009>
- Skjakbraek, G., Smidsrod, O., & Larsen, B. (1986). Tailoring of alginates by enzymatic modification invitro. *International Journal of Biological Macromolecules*, 8(6), 330–336. [https://doi.org/10.1016/0141-8130\(86\)90051-6](https://doi.org/10.1016/0141-8130(86)90051-6)
- Souto-Prieto, A., Cobos, A., Ferreira, T., Abuin-Arias, L., Rivas-Cascallar, M., & Lopez-Sanchez, P. (2025). Influence of food processing conditions on the technofunctional properties of the red seaweed Pyropia columbina. *Journal of Applied Phycology*, 37(6), 4595–4608. <https://doi.org/10.1007/s10811-025-03694-8>
- Turquois, T., & Gloria, H. (2000). Determination of the absolute molecular weight averages and molecular weight distributions of alginates used as ice cream stabilizers by using multiangle laser light scattering measurements. *Journal of Agricultural and Food Chemistry*, 48(11), 5455–5458. <https://doi.org/10.1021/jf000003v>
- Vega-Gomez, L. M., Torrego-Moreno, E., Cebrian-Lloret, V., Castanedo, M., Martinez, J. C., Recio, I., & Martinez-Sanz, M. (2026). Unravelling the multi-scale structure of cell walls from edible seaweed species and the impact of processing treatments. *Food Hydrocolloids*, 170. <https://doi.org/10.1016/j.foodhyd.2025.111720>
- Whitney, S. E. C., Brigham, J. E., Darke, A. H., Reid, J. S. G., & Gidley, M. J. (1995). In vitro assembly of cellulose/xyloglucan networks Ultrastructural and molecular aspects. *The Plant Journal*, 8(4), 491–504. <https://doi.org/10.1046/j.1365-3113.1995.8040491.x>
- Whitney, S. E. C., Brigham, J. E., Darke, A. H., Reid, J. S. G., & Gidley, M. J. (1998). Structural aspects of the interaction of mannan-based polysaccharides with bacterial cellulose. *Carbohydrate Research*, 307(3–4), 299–309. [https://doi.org/10.1016/S0008-6215\(98\)00004-4](https://doi.org/10.1016/S0008-6215(98)00004-4)
- Whitney, S. E. C., Gothard, M. G. E., Mitchell, J. T., & Gidley, M. J. (1999). Roles of cellulose and Xyloglucan in determining the mechanical properties of primary plant cell walls. *Plant Physiology*, 121(2), 657–663. <https://doi.org/10.1104/pp.121.2.657>
- Wilson, R. H., Smith, A. C., Kacuráková, M., Saunders, P. K., Wellner, N., & Waldron, K. W. (2000). The mechanical properties and molecular dynamics of plant cell wall polysaccharides studied by Fourier-transform infrared spectroscopy. *Plant Physiology*, 124(1), 397–405. <https://doi.org/10.1104/pp.124.1.397>
- Yang, L., & Zhang, L.-M. (2009). Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate Polymers*, 76(3), 349–361. <https://doi.org/10.1016/j.carbpol.2008.12.015>
- Yuk Ko, K., Pramuditha Nagahawatta, D., Lee, H.-G., Nisansala Madushani, L., Shriomala Dissanayake, D., Jeon, Y.-J., & Ko, K. Y. (2026). Navigating the challenges in fucoidan industrialization: Factors affecting biological activity and strategies for application of functional material. *Food Science and Biotechnology*, 35, 399–425. <https://doi.org/10.1007/s10068-025-01937-5>
- Zhou, L. L., Sun, D. P., Hu, L. Y., Li, Y. W., & Yang, J. Z. (2007). Effect of addition of sodium alginate on bacterial cellulose production by Acetobacter xylinum. *Journal of Industrial Microbiology & Biotechnology*, 34(7), 483–489. <https://doi.org/10.1007/s10295-007-0218-4>