

Article

Extraction Route Controls the Microstructure and Rheological Performance of Sodium Alginate from Beach-Cast *Sargassum* spp.

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Abstract

Sodium alginate was extracted from beach-cast *Sargassum* spp. collected along the coast of Puerto Progreso, Yucatán, Mexico, using two established pretreatment routes based on formaldehyde and ethanol. This study evaluates how extraction methodology controls alginate recovery, molecular structure, hydrogel rheology, macroscopic integrity, swelling behavior, and preliminary inorganic contaminant profiles. The ethanol-based route provided the highest extraction yield, reaching $19.87 \pm 0.79\%$ *w/w* for AE-5, whereas the formaldehyde route reached a maximum of $15.60 \pm 0.62\%$ *w/w* for AF-12; statistical analysis confirmed significant differences among extraction conditions (ANOVA, $p < 0.05$). Despite its lower yield, the formaldehyde route produced alginate with higher intrinsic viscosity (2.13 dL/g) and viscosity-average molecular weight (1.00×10^5 g/mol) than the ethanol-derived sample (1.33 dL/g and 0.62×10^5 g/mol), indicating better preservation of polymer chain length. ¹H NMR analysis showed that AE-5 had higher guluronic acid content ($F_G = 0.60$), lower M/G ratio (0.67), and higher G-block fraction ($F_{GG} = 0.54$), favoring Ca²⁺-mediated junction zone formation. Consequently, AE-5-derived hydrogels exhibited the highest storage modulus at 1 Hz ($G' = 23,650$ Pa), compared with AF-12-derived hydrogels (13,160 Pa) and the commercial reference (14,480 Pa). However, visual inspection and swelling analysis showed that the higher small-amplitude stiffness of AE-5 did not translate into superior macroscopic integrity; these hydrogels showed greater fragmentation during handling and higher long-term swelling. In contrast, AF-12-derived hydrogels showed lower stiffness but better apparent cohesion and a more restricted swelling profile, consistent with enhanced long-range network connectivity derived from higher molecular weight. FTIR confirmed preservation of the characteristic functional groups of sodium alginate, whereas XRD provided qualitative evidence of residual crystalline inorganic phases. Selected-metal analysis by MP-AES detected Cu in both extracted alginates, while As was detected but not quantified only in AF-12; Cd and Pb were not detected under the analytical conditions employed. Overall, the results establish a route-dependent structure-property relationship in which extraction conditions govern yield, chain preservation, block architecture, viscoelastic response, swelling behavior, and preliminary contaminant profile. These



Academic Editor: Azizur Rahman

Received: 17 April 2026

Revised: 17 June 2026

Accepted: 21 June 2026

Published: 23 June 2026

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findings support beach-cast *Sargassum* as a promising source of research-grade sodium alginate, while emphasizing that further purification, expanded contaminant profiling, arsenic speciation, biological evaluation, and direct mechanical testing are required before any food, biomedical, pharmaceutical, or environmental application can be proposed.

Keywords: sodium alginate; beach-cast sargassum; extraction route; ^1H NMR; rheology

1. Introduction

The massive accumulation of sargassum, particularly species of the *Sargassum* genus, has become a major environmental challenge along the coasts of the Caribbean Sea and the Gulf of Mexico, including the Yucatán Peninsula [1–3]. Since 2014, unprecedented and recurrent influxes of pelagic *Sargassum* spp. have been documented in the tropical Atlantic and Caribbean region, especially along the Mexican Caribbean coast [1–3]. Quantitative field and satellite-based surveys report beach-cast biomass covering surface areas ranging from approximately 21,000 to over 600,000 m² per site, with pelagic species accounting for 78–99% of the total stranded fresh biomass [2,4]. Long-term monitoring between 2016 and 2020 further indicates that *Sargassum fluitans* III represents, on average, more than 60% of the total wet biomass deposited on beaches, with pronounced interannual and seasonal variability controlling the magnitude of coastal strandings [5]. These massive strandings disrupt coastal ecosystems and severely affect economic activities such as tourism and fishing [6]. During decomposition, sargassum releases harmful compounds, including hydrogen sulfide (H₂S), ammonia (NH₃), arsenic, heavy metals, and volatile organic compounds, which degrade air and water quality, harm marine biodiversity, and pose public health risks to coastal communities [7–10]. At the same time, the recurrent arrival of large quantities of sargassum provides an abundant and locally available source of biomass that can be valorized into useful products. Among its major components, alginate is particularly attractive because of its broad technological relevance and the possibility of linking biomass management with the production of value-added polysaccharides [11–17].

Alginate is a natural anionic polysaccharide located in the cell walls of brown algae and is composed of β -D-mannuronic acid (M) and α -L-guluronic acid (G) units linked by β -(1,4) glycosidic bonds (Figure 1) [18,19]. These monomers are distributed along the polymer chain in homopolymeric (MM, GG) and heteropolymeric (MG) blocks, whose relative abundance and sequence distribution critically determine the physicochemical properties of alginate [20–23]. In particular, the gelling properties of alginate are highly sensitive to guluronic acid content, since consecutive G residues form cooperative binding sites for divalent cations such as Ca²⁺ according to the “egg-box” model [24–26]. As a result, alginate composition and block distribution strongly influence gel stiffness, elasticity, and stability, which are key parameters for many technological applications [22,23,27,28]. Importantly, these structural features are not fixed, but depend on several factors, including algal species, growth conditions, geographic origin, and processing history [16,28,29].

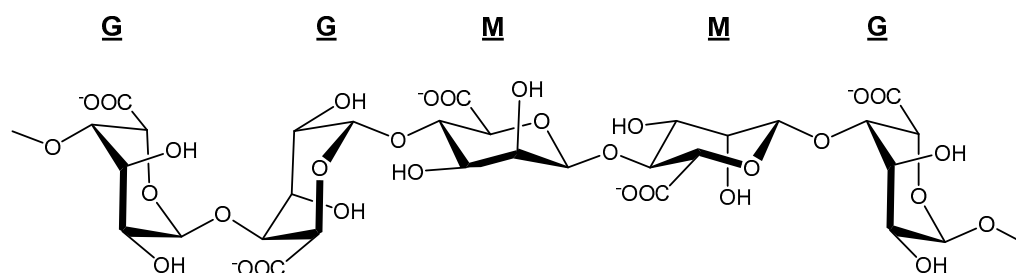


Figure 1. General structure of alginates.

Because of this structural tunability, alginate has been widely used in different technological contexts [15,30–36], including food systems, edible coatings, biodegradable packaging, encapsulation matrices, biomedical hydrogels, controlled-release platforms, and environmental sorbents [37–40]. However, the suitability of alginate for these applications cannot be assumed solely from its extraction or chemical identity. Instead, application feasibility depends on specific material attributes, including purity, molecular weight, M/G ratio, G-block distribution, viscosity, gel stiffness, swelling behavior, mechanical stability, and, where relevant, biological safety [20–29,41–45]. Therefore, in the present work, these applications are considered only as possible end-use contexts, while the experimental focus is restricted to the physicochemical, structural, and rheological parameters that may guide future application-specific evaluation. Alongside monomer composition and sequence distribution, molecular weight is another key parameter governing alginate performance [41,42]. High-molecular-weight alginates generally exhibit increased viscosity and improved gel strength, whereas lower-molecular-weight fractions may be advantageous when improved processability or controlled degradation is required [43–45]. Consequently, reliable determination of molecular weight, monomer composition, and block distribution is essential for understanding alginate functionality and for assessing its potential suitability for specific end uses [27,29,42,45].

In this context, the extraction route becomes a critical variable, since it can directly influence not only alginate yield but also the molecular characteristics that govern functional behavior. Although alternative extraction strategies such as enzyme-assisted or ionic-liquid-based methods are currently under investigation, established alkaline-acid routes remain the industrial benchmark for alginate production because of their operational simplicity and scalability [46–49]. However, even within these conventional approaches, differences in pretreatment and extraction conditions may alter polymer chain preservation, monomer distribution, and block architecture, thereby affecting the rheological and gel-forming properties of the recovered alginate [16,29,41,45]. Pretreatment strategies such as ethanol or formaldehyde exposure may affect polymer chain integrity to different extents, leading to variations in molecular weight and viscoelastic behavior. For this reason, extraction studies should not be limited to yield comparison alone but should address how processing influences the molecular structure and performance of the resulting polysaccharide.

Despite the severity of recurrent *Sargassum* strandings along the Yucatán Peninsula [1–6], the material potential of beach-cast biomass from this region remains comparatively underexplored [50]. Most previous studies on *Sargassum*-derived alginate have focused on extraction yield, general physicochemical characterization, or biomass collected from other coastal regions, particularly the Mexican Caribbean [14–16,41,45,49]. However, less attention has been paid to how conventional extraction routes modify alginate chain preservation, M/G composition, block distribution, and the resulting viscoelastic behavior of Ca^{2+} -crosslinked hydrogels [16,29,41,45,49]. This gap is relevant because alginate performance is not determined by yield alone, but by the combined effect of molecular weight, monomer sequence, and network-forming capacity [20–29,41–45]. Therefore, this

study evaluates sodium alginate extracted from beach-cast *Sargassum* spp. collected in Progreso, Yucatán, using two established pretreatment routes based on formaldehyde and ethanol [41,45]. The extracted alginates were characterized through intrinsic viscosity, viscosity-average molecular weight, ^1H NMR compositional analysis, FTIR spectroscopy, preliminary XRD screening, selected-metal analysis by MP-AES, oscillatory rheology, visual hydrogel integrity assessment, water-loss determination, and swelling analysis of Ca^{2+} -crosslinked hydrogels. Together, these analyses establish a route-dependent structure-property framework linking extraction methodology, alginate microstructure, chain preservation, Ca^{2+} -mediated hydrogel formation, macroscopic integrity, swelling behavior, and preliminary inorganic contaminant profile. This framework supports the rational selection of extraction conditions for future material development, while recognizing that further purification, safety assessment, and application-specific validation are required before beach-cast *Sargassum*-derived alginates can be proposed for end-use applications.

2. Materials and Methods

2.1. Materials

All chemical reagents were purchased from Sigma-Aldrich (Saint Louis, MO, USA), including the commercial sodium alginate used as reference material. *Sargassum* spp. biomass was collected from recently stranded seaweed on the Progreso, Yucatán, México (coordinates 21.283550, -89.677949), in October 2023. Because the biomass corresponded to beach-cast material with heterogeneous composition, it is referred to throughout as *Sargassum* spp. Prior to extraction, the biomass was washed repeatedly with tap water (ten cycles) to remove sand, plastics, and other debris. The material was subsequently rinsed with distilled water and dried at $60\text{ }^{\circ}\text{C}$ until constant weight. The dried biomass was ground and stored at room temperature in sealed plastic bags until further use.

2.2. Sodium Alginate Extraction via Formaldehyde

Sodium alginate was extracted following the procedure reported by Fertah et al. [41], with minor modifications. Briefly, 1 g of dried *Sargassum* biomass was treated with 60 mL of 2% formaldehyde solution for 30 min. The sample was then washed with distilled water ($2 \times 60\text{ mL}$) and filtered. An acid pretreatment was performed by immersing the solid in 60 mL of a 0.2 M hydrochloric acid solution under variable time conditions (1 and 2 h), followed by filtration and washing with distilled water. Alkaline extraction was performed by placing the treated biomass in 60 mL of 2% sodium carbonate solution under variable time (1, 2, and 3 h) and temperature (70 and $50\text{ }^{\circ}\text{C}$) conditions. The resulting solution was filtered to remove residual biomass. Finally, 180 mL of 96% ethanol was added to the sample in a 1:3 (*v/v*) ratio, maintaining a temperature of $5\text{ }^{\circ}\text{C}$, promoting the precipitation of sodium alginate as fibrous material. The precipitated alginate fibers were separated, and the recovered supernatant was discarded. The alginate fibers were then washed with ethanol (20 mL) and acetone (20 mL) to remove residual impurities and finally dried in an oven at $80\text{ }^{\circ}\text{C}$ until constant weight was achieved. Extraction yield (%) was calculated as the mass of dried sodium alginate recovered relative to the initial dry biomass mass. All extraction experiments were performed in triplicate.

2.3. Sodium Alginate Extraction via Ethanol

Sodium alginate extraction using ethanol was carried out following the methodology described by Lorbeer et al. [45], with slight modifications. Dried *Sargassum* biomass (1 g) was treated with 60 mL of 96% ethanol under magnetic stirring (400 rpm) for 3 h. The sample was then washed with distilled water, filtered, and dried at $80\text{ }^{\circ}\text{C}$ for 12 h. Next, an acid pretreatment was performed by immersing the biomass in 60 mL of 0.2 M hydrochloric

acid solution at 50 °C under variable time conditions (1.5, 2.0, 2.5, and 3.0 h). The solid was subsequently filtered and washed with distilled water. Alkaline extraction was carried out by treating the residual biomass with 60 mL of 2% sodium carbonate solution at 45 °C under variable time conditions (1, 2, or 3 h). After filtration to remove insoluble residues, the solution volume was adjusted to 100 mL with distilled water, and sodium alginate was precipitated by adding 300 mL of 96% ethanol (1:3 (*v/v*) ratio). The precipitate was washed with ethanol (20 mL) and acetone (20 mL) and dried at 80 °C until constant weight. Representative photographs documenting the precipitation and recovery of sodium alginate are provided in the Supplementary Information (Figure S18). Extraction yield (%) was calculated as the mass of dried sodium alginate recovered relative to the initial dry biomass mass. All extraction experiments were performed in triplicate.

2.4. Solution Preparation, Rheological Measurements, and Hydrogel Swelling Analysis

Sodium alginate solutions were prepared at concentrations ranging from 0.1 to 0.7% (*w/v*) in 0.1 M NaCl for intrinsic viscosity and molecular weight estimation. The NaCl solution was heated to 50 °C, and sodium alginate was gradually added under continuous stirring at 300 rpm for 2 h until complete dissolution. The solutions were further stirred during cooling to 25 °C and used immediately after preparation. Rheological measurements of sodium alginate solutions were conducted at 25 °C using a TA Discovery HR-3 hybrid rheometer (TA Instruments, New Castle, DE, USA) equipped with a Peltier temperature control system. A cone-and-plate geometry (40 mm diameter, 0.5081° cone angle, 17 µm gap) was employed, using approximately 150 µL of sample. Flow measurements were carried out over a shear rate range of 0.1–1000 s^{−1}. All samples exhibited non-Newtonian behavior characterized by shear-thinning. Apparent viscosity values were obtained by linear extrapolation of the low-shear-rate region and were subsequently used to calculate the specific viscosity of dilute alginate solutions for intrinsic viscosity determination. Measurements were performed in triplicate.

Hydrogels were prepared from sodium alginates extracted via both routes to evaluate their viscoelastic properties. A 1% (*w/v*) sodium alginate solution was homogenized by stirring at 300 rpm and 50 °C for 2 h and then allowed to cool gradually to 25 °C under continuous stirring. Subsequently, 1 mL of alginate solution was transferred to a cylindrical mold, and crosslinking was initiated by adding 1 mL of 0.1 M CaCl₂ solution (1:1 volume ratio). This initial calcium concentration was used to promote gradual ionotropic gelation and reduce the formation of highly heterogeneous crosslinked domains [51,52]. The mixture was allowed to gel at room temperature for 1 h, after which excess liquid was removed. To further consolidate the gel network, 1 mL of 3 M CaCl₂ solution was then added, and crosslinking was allowed to proceed for an additional 1 h [53]. This two-step protocol was used to balance initial gel homogeneity and final network consolidation, while avoiding the immediate surface over-gelation that may occur upon direct exposure to highly concentrated calcium solutions [54]. Since the same crosslinking protocol was applied to all alginate samples, the resulting hydrogels were used for comparative evaluation of the effect of alginate source and molecular structure on viscoelastic behavior. After removal of residual liquid, the hydrogels were immediately subjected to rheological analysis.

Hydrogel rheological measurements were performed using a Kinexus Pro+ rotational rheometer (Malvern Panalytical Ltd., Malvern, UK) equipped with a Peltier temperature control system. A cone-and-plate geometry (40 mm diameter, 4.0107° cone angle, 0.1473 mm gap) was used at 25 °C. The linear viscoelastic region (LVR) was determined by strain sweep experiments conducted at a fixed frequency of 1 Hz. Frequency sweep tests were subsequently carried out within the LVR over a frequency range of 0.1–10 Hz using the strain value identified from the preceding strain sweep tests.

Hydrogel swelling behavior was evaluated gravimetrically to assess water uptake and network stability. Freshly prepared hydrogels were first weighed immediately after gelation to obtain the initial wet mass (W_0). The samples were then dried at 60 °C until constant weight, and the dry mass (W_d) was recorded. Water loss during drying (W_L) was calculated according to the following equation:

$$W_L(\%) = \frac{w_0 - w_d}{w_0} \times 100$$

For swelling measurements, the dried hydrogels were immersed in deionized water at room temperature. At defined time intervals, the samples were removed from the medium, gently blotted with filter paper to remove excess surface water, and weighed (W_t). This procedure was repeated until equilibrium swelling was reached, as indicated by negligible changes in mass between consecutive measurements. The swelling ratio (S) was calculated using the following equation:

$$S(\%) = \frac{w_t - w_d}{w_d} \times 100$$

All swelling and water-loss measurements were performed in triplicate using independently prepared hydrogels, and results are reported as mean $\pm$ standard deviation.

2.5. Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis

^1H NMR experiments were performed using a JEOL ECA-500 NMR spectrometer, (JEOL Ltd., Tokyo, Japan) and spectra were processed using MestReNova software (version 12.0.0, Mestrelab Research, Santiago de Compostela, A Coruña, Spain). Measurements were carried out at 500 MHz and 80 °C. For each sample, spectra were recorded at three temperatures: 20 °C (before heating), 80 °C, and 20 °C (after cooling) to assess thermal stability and signal reproducibility. Only spectra acquired at 80 °C are reported, as they provided optimal signal resolution for structural characterization. Spectra used for quantitative comparison were acquired and processed under identical experimental conditions.

Sodium alginate samples were dissolved in D_2O at a concentration of 1% (w/v). A presaturation technique was applied to suppress the residual water signal and improve spectral resolution. Chemical shift assignments and experimental conditions followed the ASTM F2259-10 (2012) standard [55]. Peak assignments were as follows: the anomeric proton of guluronic acid (signal A) at 5.07 ppm; H-5 protons of central guluronic acid residues in GGM and MGM triads (signals B1 and B2) at 4.76 and 4.73 ppm, respectively; anomeric protons of mannuronic acid residues adjacent to guluronic (signal B3) or mannuronic residues (signal B4) at 4.70 and 4.67 ppm; and the H-5 proton of guluronic acid (signal C) at 4.46 ppm.

Peak deconvolution and integration were performed in MestReNova (Version 17.0.0) to resolve overlapping signals in the anomeric region, enabling accurate quantification of guluronic and mannuronic acid fractions, as well as dyad and triad block distributions.

2.6. Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectra were recorded using an Agilent Cary 630 infrared spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with an attenuated total reflectance (ATR) accessory. Measurements were conducted at room temperature (25 °C) over the spectral range of 500–4000 cm^{-1} . Spectra were collected directly from solid samples placed on the ATR crystal.

2.7. Preliminary Inorganic Residue Screening and Selected Metal Analysis

Preliminary screening of crystalline inorganic residues in the extracted alginates was performed by X-ray diffraction (XRD). XRD patterns were recorded using a Bruker D8 Advance diffractometer (Bruker AXS SE, Karlsruhe, Germany) equipped with a Cu $K\alpha_1$ radiation source, operating at 40 kV and 30 mA. Measurements were performed at room temperature with a step size of 0.02° and a counting time of 0.5 s per step, over a 2θ range of $5\text{--}80^\circ$. Samples were analyzed in solid form and mounted on a flat sample holder for data acquisition. The XRD patterns were used only as qualitative screening evidence for crystalline residual phases and are provided in the Supporting Information File (Figure S19).

Selected metal analysis was performed for As, Cd, Cu, and Pb in ethanol-derived alginate (AE-5) and formaldehyde-derived alginate (AF-12) by microwave plasma atomic emission spectroscopy (MP-AES; Agilent 4200, Agilent Technologies, Inc. Santa Clara, CA, USA) after acid digestion. For digestion, 75 mg of each dried alginate sample was placed in a digestion tube with 500 μL of concentrated HNO_3 . The tubes were heated at 100°C for 8 h, cooled to room temperature, and diluted with deionized water to a final volume of 1.5 mL prior to analysis. The results were expressed as mg kg^{-1} of dry alginate. This analysis was intended as a preliminary selected-metal assessment and not as a complete contaminant or safety evaluation.

2.8. Statistical Analysis

Statistical analysis was performed to evaluate the significance of differences in extraction yields among the experimental conditions tested for both extraction routes. All data are reported as mean $\pm$ standard deviation of three independent experiments. A one-way analysis of variance (ANOVA) was applied separately to each extraction route to determine whether statistically significant differences existed among the evaluated conditions. When significant differences were detected ($p < 0.05$), Tukey's post hoc test was used for multiple comparisons between means. The level of significance was set at $\alpha = 0.05$. Statistical analyses were carried out using OriginPro 2021 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. Sodium Alginate Extraction: Study and Yield Analysis

Sodium alginate was successfully extracted from beach-cast *Sargassum* spp. using formaldehyde- and ethanol-based routes under the experimental conditions summarized in Tables 1 and 2. In general, the ethanol-based route afforded higher extraction yields, whereas the formaldehyde-based route was later found to better preserve polymer chain length, indicating that extraction performance should not be assessed solely in terms of yield. The obtained yields varied across extraction routes and experimental conditions, indicating the influence of treatment time and temperature during the acidic and alkaline stages.

For the formaldehyde route, the highest yield was obtained for sample AF-12 (15.60% w/w), followed by AF-3 (10.91%) and AF-5 (8.41%). In the case of AF-12, extraction was carried out using a relative short alkaline treatment time (1 h) at 50°C , indicating that prolonged exposure to alkaline conditions or higher temperatures does not necessarily enhance alginate recovery. In contrast, samples such as AF-4 and AF-11 exhibited lower yields (4.40 and 2.39%, respectively), which can be associated with less favorable combinations of treatment time and temperature during the extraction stages.

Table 1. Extraction parameters using the formaldehyde route.

Sample	0.2 M HCl (Time, h)	2% Sodium Carbonate (Time, h)	0.2 M Sodium Carbonate (Temp., °C)	Yield (% <i>w/w</i>) *
AF-1	2	3	70	6.66 ± 0.27
AF-2	2	2	70	6.06 ± 0.24
AF-3	2	1	70	10.91 ± 0.44
AF-4	1	3	70	4.40 ± 0.18
AF-5	1	2	70	8.41 ± 0.34
AF-6	1	1	70	5.61 ± 0.22
AF-7	2	3	50	7.27 ± 0.29
AF-8	2	2	50	7.05 ± 0.28
AF-9	2	1	50	6.18 ± 0.25
AF-10	1	3	50	8.89 ± 0.36
AF-11	1	2	50	2.39 ± 0.10
AF-12	1	1	50	15.60 ± 0.62

* All experiments were performed in triplicate, and values are reported as mean ± standard deviation.

Table 2. Extraction parameters using the ethanol route.

Sample	0.2 M HCl (Time, h)	0.2 M HCl (Temp., °C)	2% Sodium Carbonate (Time, h)	0.2 M Sodium Carbonate (Temp., °C)	Yield (% <i>w/w</i>) *
AE-1	3	50	2	45	15.37 ± 0.61
AE-2	2.5	50	2	45	14.39 ± 0.58
AE-3	2	50	2	45	12.67 ± 0.51
AE-4	1.5	50	2	45	11.19 ± 0.45
AE-5	3	50	3	45	19.87 ± 0.79
AE-6	2.5	50	3	45	16.65 ± 0.67
AE-7	2	50	3	45	11.00 ± 0.44
AE-8	1.5	50	3	45	14.43 ± 0.58
AE-9	3	50	1	45	13.87 ± 0.55
AE-10	2.5	50	1	45	10.53 ± 0.42
AE-11	2	50	1	45	12.26 ± 0.49
AE-12	1.5	50	1	45	6.97 ± 0.28

* All experiments were performed in triplicate, and values are reported as mean ± standard deviation.

A statistical evaluation of the extraction yields obtained via the formaldehyde route revealed significant differences among the experimental conditions (one-way ANOVA, $F_{11,24} = 335.12$, $p < 0.001$). Post hoc analysis using Tukey's test confirmed that several pairwise comparisons were statistically different ($p < 0.05$), indicating that the extraction parameters strongly influence alginate recovery. In particular, the treatment AF-12 exhibited the highest yield within the formaldehyde route, and Tukey's test showed that this condition was statistically differentiated from several lower-yielding treatments. The magnitude of the difference relative to the experimental variability supports the interpretation that the enhanced performance of AF-12 is associated with the specific combination of mild temperature (50 °C) and reduced alkaline treatment time (1 h), rather than random variation.

The ethanol-based route generally produced higher yields than the formaldehyde method. The maximum yield was obtained for sample AE-5 (19.87% *w/w*), corresponding to extended acidic (3 h at 50 °C) and alkaline (3 h at 45 °C) treatment times. Samples AE-6 (16.65%) and AE-1 (15.37%) also showed relatively high yields, whereas AE-12 (6.97%) and AE-10 (10.53%) exhibited lower extraction efficiencies. Because temperature was kept constant in the ethanol route, the observed variations in yield primarily reflect the influence of treatment duration, particularly during the alkaline extraction step. This behavior is consistent with reduced polymer degradation during extraction, which favors the preservation of alginate chains.

A similar statistical trend was observed for the ethanol-based extraction route. One-way ANOVA confirmed the presence of significant differences among the tested conditions ($F_{11,24} = 113.7$, $p < 0.001$), highlighting the effect of treatment time on extraction efficiency. Tukey's multiple comparison test indicated that AE-5, the highest-yielding condition within the ethanol route, was statistically differentiated from several lower-yielding treatments. The superior yield obtained for AE-5 ($19.87 \pm 0.79\%$ *w/w*) can therefore be attributed to a consistent and reproducible effect of prolonged acid and alkaline treatment times, rather than experimental variability.

The yields obtained in this study are comparable to those reported in the literature for *Sargassum* and other brown algae species (Table 3). For example, alginate yields of approximately 16.9% have been reported for *Sargassum vulgare* using formaldehyde-based extraction, while higher values have been achieved for species such as *Nizimuddinina zanardini* (24.0%) and *Macrocystis pyrifera* (23.15%) under more aggressive processing conditions. Importantly, the yields obtained here were achieved without resorting to extended reaction times or elevated temperatures.

Table 3. Comparison of extraction techniques and yields reported in the literature.

Macroalgae Species	Extraction Conditions	Yield (%)	References
<i>Sargassum vulgare</i> (SVHV)	2% formaldehyde (24 h), 0.2 M HCl (24 h), 2% Na ₂ CO ₃ (5 h at 60–80 °C)	16.90	Torres et al. [56]
<i>Sargassum vulgare</i> (SVLV)	2% formaldehyde (24 h), 0.2 M HCl (24 h), 2% Na ₂ CO ₃ (5 h at 60–80 °C)	16.90	Torres et al. [56]
<i>Nizimuddinina zanardini</i>	2% formaldehyde (24 h), 0.2 M HCl (3 h at 60 °C), 3% Na ₂ CO ₃ (2.5 h at 60 °C)	24.00	Khajouei et al. [29]
<i>Macrocystis pyrifera</i>	0.2% formaldehyde (overnight), 0.1 N HCl (30 min), 0.5% Na ₂ CO ₃ (1 h at 60 °C)	23.15	Gao et al. [57]
<i>Sargassum natans</i>		23.00	
<i>Sargassum vulgare</i>		17.00	
<i>Padina gymnospora</i>	2% formaldehyde (overnight), 0.2 M HCl, 2% Na ₂ CO ₃ (3 h at 99 °C)	16.00	Rhein-Knudsen et al. [15]
<i>Padina antillarum</i>		22.00	
<i>Laminaria digitate</i>		29.00	
<i>Macrocystis pyrifera</i>		26.00	

Table 3. Cont.

Macroalgae Species	Extraction Conditions	Yield (%)	References
Waste <i>Sargassum natans</i>	2% formaldehyde (overnight), 5% Na ₂ CO ₃ (15:1 alkali:alga ratio, 2 h at 65 °C)	15.00	Mohammed et al. [58]
<i>Durvillaea antarctica</i>	2% formaldehyde (24 h), 0.2 M HCl (3 h at 60 °C), 3% Na ₂ CO ₃ (2.5 h at 60 °C)	20.80	Caballero et al. [59]
<i>Ascophyllum nodosum</i>	0.2 M HCl (12 h at RT),	13.80	Bojorgues et al. [49]
<i>Saccharina latissima</i>	0.1 M NaHCO ₃ (2 h at RT)	11.20	
<i>Sargassum turbinaroides</i>	2% formaldehyde (24 h at 90 °C), 0.2 M HCl (24 h), 2% Na ₂ CO ₃ (3 h at 100 °C)	10.00	Laroche et al. [60]
<i>Cystoseira barbata</i>	0.1 M HCl (2 h at 60 °C), 3% Na ₂ CO ₃ (2 h at 60 °C)	9.90	Sellimi et al. [61]

An important aspect of the present work is the use of beach-cast *Sargassum* biomass as the feedstock. Despite being classified as residual material, the alginate yields obtained using the ethanol-based route, particularly for sample AE-5 (19.87% *w/w*), are comparable to those reported for marine-harvested species traditionally exploited for alginate production, such as *Sargassum natans* (23%) and *Durvillaea antarctica* (20.8%). Moreover, the yields reported here exceed those reported for species such as *Saccharina latissima* (11.2%) and *Sargassum turbinaroides* (10%).

These results indicate that both extraction routes allow effective recovery of sodium alginate from stranded *Sargassum*. The formaldehyde-based method affords moderate to high yields under relatively mild conditions, whereas the ethanol-based route enables higher recoveries under controlled treatment times while limiting excessive processing. However, the relevance of each route cannot be judged on yield alone, since extraction methodology also influences the molecular features that govern the functional behavior of the recovered alginates. Accordingly, the choice of extraction route should be guided by the intended application and processing constraints rather than by yield considerations alone.

The selection of samples AE-5 and AF-12 for detailed NMR and rheological characterization was based on extraction yield performance and statistical comparison among extraction conditions. Both samples exhibited the highest yields within their respective extraction routes (19.87% *w/w* for AE-5 and 15.60% *w/w* for AF-12). One-way ANOVA confirmed significant differences among extraction conditions within each route ($p < 0.05$), and Tukey's post hoc test supported the statistical differentiation of these high-yielding conditions from several lower-yielding treatments. In addition, these samples represent distinct extraction regimes in terms of treatment duration and severity, allowing a representative comparison of the influence of extraction route on the molecular structure and viscoelastic behavior of the resulting alginates.

3.2. Rheological Studies

Because alginate performance depends not only on extraction yield but also on chain conformation and molecular size, rheological analysis of dilute solutions and crosslinked hydrogels was used to evaluate the functional consequences of the extraction route. Intrinsic viscosity and viscosity-average molecular weight were first determined as indirect descriptors of polymer chain dimensions in solution, and the resulting alginates were

subsequently examined as Ca^{2+} -crosslinked hydrogels to establish how these molecular differences translated into viscoelastic behavior.

3.2.1. Intrinsic Viscosity

Intrinsic viscosity is a key parameter for alginate characterization, as it is directly related to polymer conformation in solution and governs macroscopic rheological behavior. The use of saline media for alginate characterization is well established, as ionic strength modulates electrostatic interactions between carboxylate groups along the polymer backbone. Early studies by Smidsrød and Haug showed that increased ionic strength screens intramolecular electrostatic repulsions, resulting in more compact chain conformations [62]. Subsequent work by Martinsen et al. confirmed the influence of the ionic environment on the physicochemical properties of alginate solutions [63]. More recently, Storz et al. showed that NaCl-containing media improve the reliability of intrinsic viscosity determinations by suppressing long-range electrostatic interactions [64,65]. Accordingly, a NaCl concentration of 0.1 M was selected in this work as a compromise between effective charge screening and preservation of polymer structure, enabling direct comparison with previously reported data [66,67].

Intrinsic viscosity, $[\eta]$, is defined as the limiting ratio of specific viscosity to polymer concentration in the dilute-solution limit [68]:

$$[\eta] = \lim_{c \rightarrow 0} \frac{\eta_{sp}}{c} \quad (1)$$

where η_{sp} is the specific viscosity and c is the polymer concentration [62]. Under dilute solution conditions, $[\eta]$ can be obtained experimentally by extrapolating $\frac{\eta_{sp}}{c}$ to zero concentration. To facilitate such extrapolation, the Huggins equation is commonly used:

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \quad (2)$$

where k_H is the Huggins constant. This linear relationship between $\frac{\eta_{sp}}{c}$ and c allows $[\eta]$ to be determined as the intercept of a linear least-squares fit (Figure 2).

Figure 2 shows representative linear fits for commercial sodium alginate (CSA), and for alginates extracted via the formaldehyde (AF-12) and ethanol (AE-5) routes. The intrinsic viscosity values determined were 1.93 dL/g for CSA, 2.13 dL/g for AF-12, and 1.33 dL/g for AE-5. All regressions exhibited coefficients of determination (R^2) greater than 0.99, indicating excellent linearity.

The alginate extracted via the formaldehyde route (AF-12) exhibited a higher intrinsic viscosity (2.13 dL/g) than the commercial reference (1.93 dL/g), consistent with the preservation of longer polymeric chains during extraction. In contrast, the ethanol-based alginate (AE-5) displayed a lower intrinsic viscosity (1.33 dL/g), consistent with shorter average chain lengths.

Comparison with literature values provides additional context for the quality of the extracted alginates (Table 4). Torres et al. [56] reported intrinsic viscosity values of 6.9 and 4.1 dL/g for *Sargassum vulgare*, which are higher than those obtained in the present study. Such differences are commonly attributed to variations in algal species, harvest season, geographic origin, and environmental growth conditions. The intrinsic viscosity obtained for AF-12 (2.13 dL/g), although lower than these values, falls within the range reported for other brown algae, such as *Sargassum cristaefolium* (4.47 dL/g) [69] and *Cystoseira barbata* (4.06 dL/g) [70].

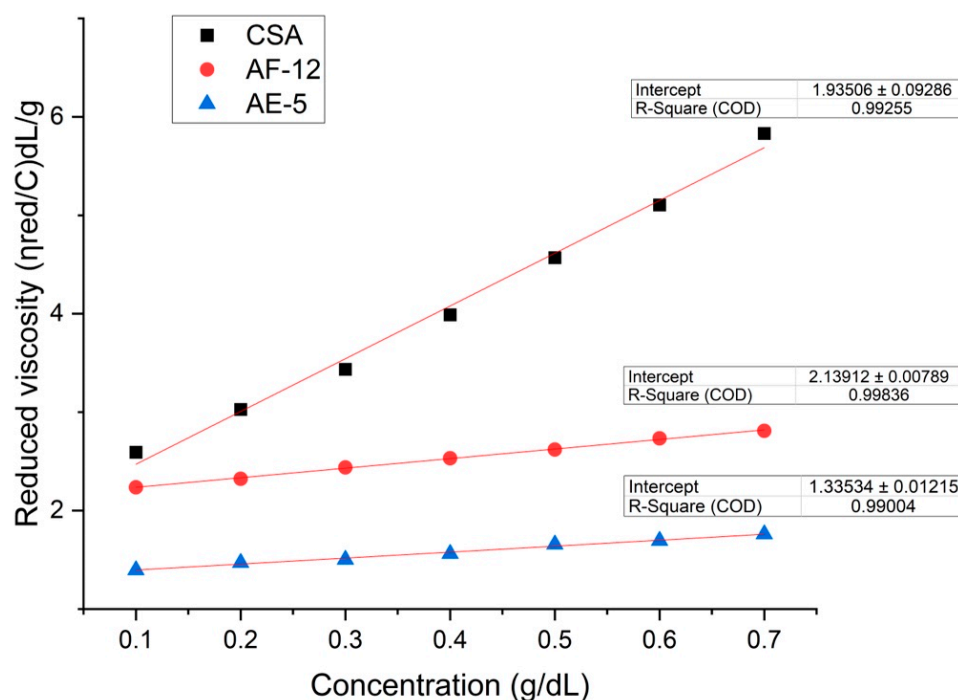


Figure 2. Linear fit to determine the intrinsic viscosity of commercial sodium alginate (CSA), AE-5, and AF-12 samples.

Although the ethanol-based extraction yielded alginates with a lower intrinsic viscosity (1.33 dL/g), this value remains comparable to that of the commercial reference material and lies within a range relevant for practical and industrial applications. Overall, these results indicate that the formaldehyde route better preserves chain dimensions in solution, whereas the ethanol route yields alginates with lower intrinsic viscosity but still within a range compatible with functional use.

3.2.2. Estimation of Viscosity-Average Molecular Weight

The viscosity-average molecular weight (M_v) was estimated using the Mark–Houwink equation,

$$[\eta] = kM_v^a \quad (3)$$

where k and a are empirical constants dependent on the polymer-solvent system [62]. This equation provides a practical link between intrinsic viscosity and an apparent molecular weight derived from solution behavior [71].

Intrinsic viscosity was correlated with molecular weight using an empirical relationship reported by Clementi et al. [72]:

$$[\eta] = 0.023M^{0.984} \quad (4)$$

where M represents an apparent molecular weight derived from intrinsic viscosity measurements. It should be noted that this approach provides an estimation related to the viscosity-average molecular weight (M_v), rather than a direct determination of absolute weight-average molecular weight (M_w).

Using this approach, viscosity-average molecular weight values of 0.94×10^5 g/mol for CSA, 1.00×10^5 g/mol for AF-12, and 0.62×10^5 g/mol for AE-5 (Table 4).

Table 4. Intrinsic viscosity and average molecular weight of alginates from different biomass sources.

Macroalgae Species	Intrinsic Viscosity (dL/g)	Estimated Viscosity-Average Molecular Weight, M_v ($\times 10^5$ g/mol)	References
<i>Sargassum vulgare</i> (SVHV)	6.9	3.30	Torres et al. [56]
<i>Sargassum vulgare</i> (SVLV)	4.1	1.94	Torres et al. [56]
<i>Nizimuddinina zanardini</i>	3.42	1.03	Khajouei et al. [29]
<i>Sargassum natans</i>	-	5.69	Rhein-Knudsen et al. [15]
<i>Sargassum vulgare</i>	-	5.14	
<i>Padina gymnospora</i>	-	4.82	
<i>Laminaria digitate</i>	-	7.56	
<i>Macrocystis pyrifera</i>	-	7.19	
<i>Cystoseira barbata</i>	2.83	2.04	Sellimi et al. [61]
<i>Sargassum cristaefolium</i>	4.47	2.11	Sugiono et al. [69]
<i>Cystoseira barbata</i>	4.06	1.26	Trica et al. [70]
<i>Sargassum vulgare</i>	-	1.10	Sari et al. [73]
<i>Sargassum</i> spp. (Formaldehyde)	2.13 ± 0.0079 *	1.00 ± 0.004 *	This study
<i>Sargassum</i> spp. (Ethanol)	1.33 ± 0.012 *	0.62 ± 0.006 *	
Commercial sodium alginate	1.93 ± 0.093 *	0.94 ± 0.044 *	

* All experiments were performed in triplicate, and values are reported as mean $\pm$ standard deviation.

The M_v values obtained via the formaldehyde route (1.00×10^5 g/mol) are comparable to that reported by Khajouei et al. [29], for *Nizimuddinina zanardini* (1.03×10^5 g/mol). In contrast, the ethanol-based route yielded alginates with a lower molecular weight (0.62×10^5 g/mol), which nevertheless falls within a range comparable to that of the commercial sodium alginate used as reference (0.94×10^5 g/mol). Although these values are lower than those reported for brown algae such as *Macrocystis pyrifera* (7.19×10^5 g/mol) or *Laminaria digitata* (7.56×10^5 g/mol), they are comparable to those reported by Sari et al. [73], for *Sargassum vulgare* (1.10×10^5 g/mol).

Thus, the viscosity-average molecular weight data reinforce the idea that the extraction route governs not only alginate recovery, but also the chain-length distribution that later influences gel formation and mechanical response.

3.2.3. Viscoelastic Properties, Macroscopic Integrity, and Swelling Behavior of Hydrogels

To evaluate how route-dependent differences in alginate structure affected hydrogel performance, oscillatory rheological measurements were performed on Ca^{2+} -crosslinked hydrogels prepared from AE-5, AF-12, and commercial sodium alginate (CSA). Strain sweep experiments conducted at a fixed frequency of 1 Hz were first used to determine the linear viscoelastic region (LVR), which extended up to 0.1% strain for all samples. Frequency sweep tests were then carried out within this regime over a frequency range of 0.1–10 Hz. The corresponding strain sweep profiles are provided in the Supplementary Information File (Figure S1). As shown in Figure 3, all hydrogel samples exhibited storage moduli (G') higher than loss moduli (G'') across the entire frequency range, confirming their gel-like behavior ($\tan \delta = G''/G' < 1$). At 1 Hz, the hydrogel derived from AE-5 (HAE) displayed

the highest storage modulus ($G' = 23,650$ Pa), followed by the commercial alginate hydrogel (HAS, $G' = 14,480$ Pa) and the formaldehyde-derived hydrogel (HAF, $G' = 13,160$ Pa). The corresponding loss moduli followed the same trend, with values of 3090 Pa for HAE, 2394 Pa for HAS, and 1624 Pa for HAF. Both G' and G'' showed weak positive frequency dependence, consistent with physically crosslinked polymer networks [74–78].

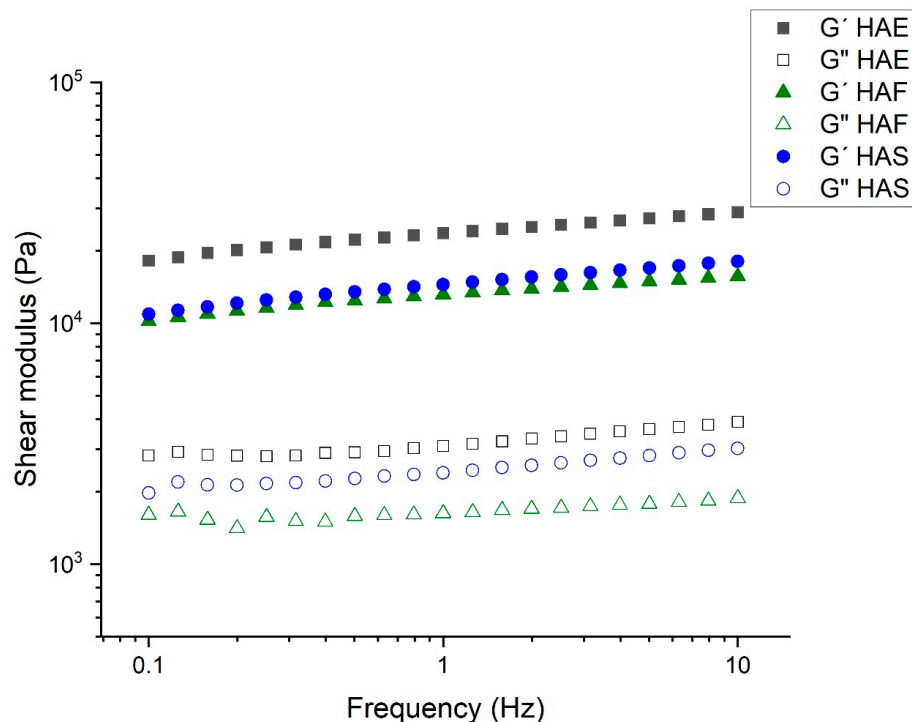


Figure 3. Frequency dependence of the storage (G') and loss (G'') moduli for hydrogels derived from alginates extracted via the ethanol route (HAE), formaldehyde route (HAF), and commercial sodium alginate (HAS).

The higher G' value observed for HAE indicates that, under small-amplitude oscillatory deformation, this hydrogel forms the stiffest network among the evaluated samples. This behavior is consistent with the higher guluronic acid fraction and G-block content of AE-5, which favor the formation of Ca^{2+} -mediated junction zones according to the egg-box model [79]. However, oscillatory rheology within the LVR reflects the response of the network under small deformation and does not necessarily predict macroscopic handling integrity, fracture resistance, or dimensional stability under larger deformation. To provide an additional comparative descriptor of small-amplitude elastic stiffness, the apparent Young's modulus (E_{app}) was estimated from the storage modulus obtained at 1 Hz. Assuming nearly incompressible behavior for highly hydrated hydrogels ($\nu \approx 0.5$), the relationship $E_{\text{app}} \approx 3G'$ was applied [80–82]. Based on this approximation, HAE exhibited the highest E_{app} value (~ 70.9 kPa), followed by HAS (~ 43.4 kPa) and HAF (~ 39.5 kPa). These values should not be interpreted as true static compressive moduli, because no direct compression test was performed. Instead, they provide an indirect comparative parameter for small-amplitude elastic stiffness under the oscillatory conditions used in this study [83–85].

Although HAE showed the highest storage modulus and estimated E_{app} , visual inspection revealed lower macroscopic integrity during handling. To document this behavior, representative photographs of HAE, HAF, and HAS, were recorded at different stages: immediately after gelation, after demolding/handling, and after drying. As shown in Figure 4, HAE displayed visible fragmentation after demolding, whereas HAF and HAS retained

more continuous structures under the same handling conditions. After drying, HAE also showed greater structural collapse, while HAF and HAS maintained more defined shapes. These observations are qualitative and should not be considered a substitute for direct mechanical testing; however, they provide visual evidence that the highest small-amplitude stiffness does not necessarily correspond to the best macroscopic cohesion.

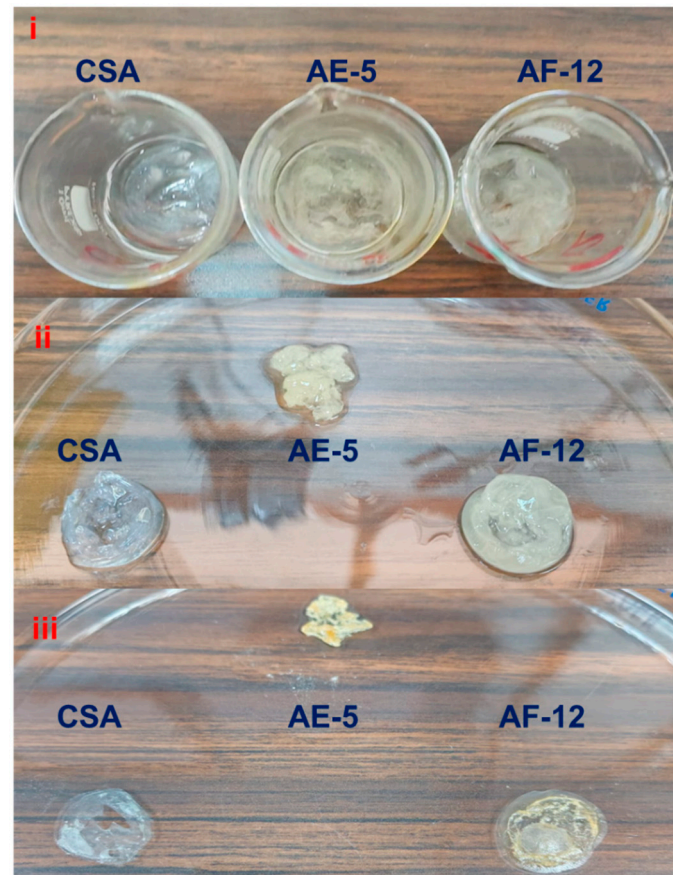


Figure 4. Macroscopic appearance and handling integrity of Ca^{2+} -crosslinked hydrogels derived from ethanol-extracted alginate (HAE), formaldehyde-extracted alginate (HAF), and commercial sodium alginate (HAS): (i) immediately after gelation, (ii) after demolding/handling, and (iii) after drying.

The apparent discrepancy between the high G' value of HAE and its lower handling integrity highlights the multiscale nature of hydrogel behavior. The storage modulus reflects the elastic response of the crosslinked network under small oscillatory deformation, whereas macroscopic cohesion depends on additional factors such as chain length, chain entanglement, network continuity, and resistance to crack or fracture propagation [83,85]. In this sense, the lower viscosity-average molecular weight of AE-5 may limit long-range chain connectivity, promoting the formation of locally stiff Ca^{2+} -crosslinked domains but reducing the ability of the hydrogel to maintain a continuous structure during handling. In contrast, the higher molecular weight of AF-12 may favor longer-range connectivity and network continuity, even though HAF showed a lower storage modulus than HAE. To complement the visual assessment, water loss during drying and swelling behavior were evaluated gravimetrically. Water loss during drying provides an estimate of the water retained in the freshly formed hydrogels prior to drying. As shown in Table 5, all hydrogels exhibited high water content, with water loss values ranging from approximately 86% to 89%. HAF showed the highest water loss during drying ($89.42 \pm 5.96\%$), followed by HAS ($87.39 \pm 6.39\%$) and HAE ($86.38 \pm 6.66\%$). These values, reported as mean $\pm$ standard

deviation from triplicate measurements, confirm the highly hydrated nature of the Ca^{2+} -crosslinked alginate networks.

Table 5. Water loss during drying of Ca^{2+} -crosslinked hydrogels relative to the freshly formed state.

Hydrogel	Fresh Mass (mg) *	Dry Mass (mg) *	Water Loss During Drying, WL (%) *
HAS	626.2 ± 26.5	78.9 ± 4.7	87.39 ± 6.39
HAF	905.7 ± 29.8	95.8 ± 5.6	89.42 ± 5.96
HAE	461.1 ± 18.9	62.8 ± 4.1	86.38 ± 6.66

* All experiments were performed in triplicate, and values are reported as mean $\pm$ standard deviation.

Swelling analysis provided additional quantitative information on water uptake and network stability after drying and rehydration. Swelling values are reported as mean $\pm$ standard deviation from triplicate measurements. As shown in Figure 5, the hydrogels exhibited distinct swelling profiles depending on the alginate source. HAS reached a maximum swelling value of approximately 163.4% at 120 min, followed by a gradual decrease and stabilization at longer times. HAF showed a more restricted swelling profile, reaching a maximum value of approximately 115.9% at 210 min. This lower swelling response suggests a more constrained network structure under the conditions evaluated. In contrast, HAE exhibited a delayed but more pronounced increase in swelling, reaching values above 190% at longer times.

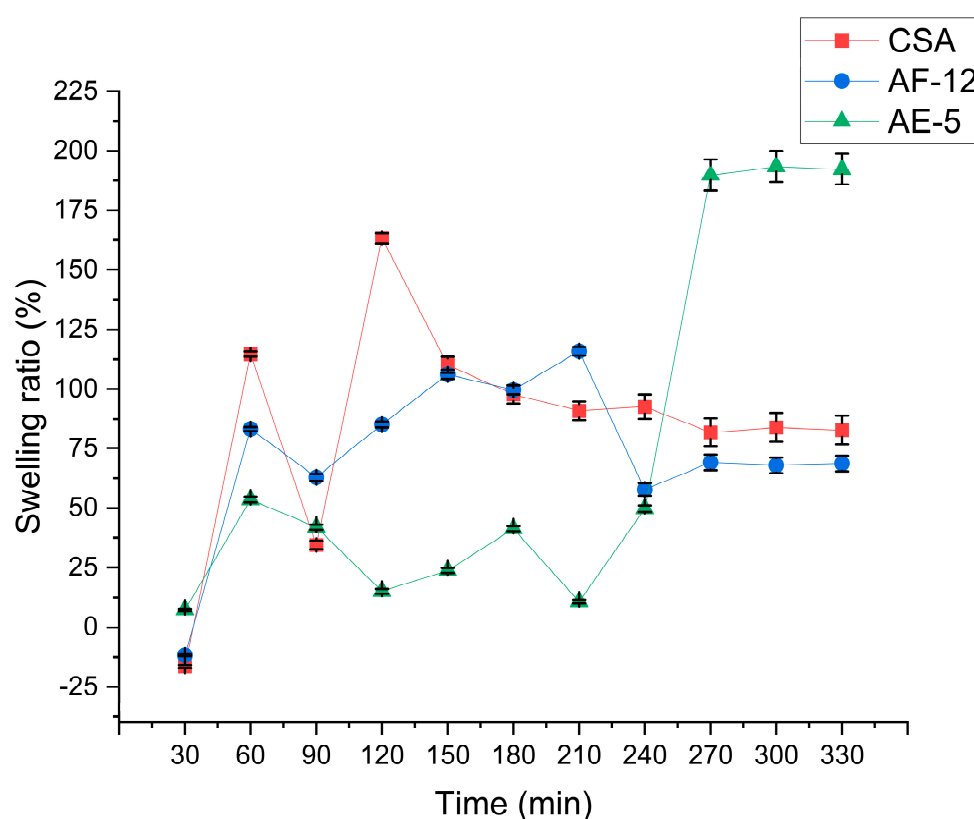


Figure 5. Swelling ratio (%) as a function of time for Ca^{2+} -crosslinked hydrogels derived from ethanol-extracted alginate (HAE), formaldehyde-extracted alginate (HAF), and commercial sodium alginate (HAS). Data are reported as mean $\pm$ standard deviation ($n = 3$).

The higher long-term swelling observed for HAE is consistent with its reduced macroscopic integrity during handling. Although AE-5 contains a higher fraction of guluronic acid and G-rich blocks, which favors local Ca^{2+} -mediated crosslinking and produces a

high G' value, its lower molecular weight may reduce chain entanglement and long-range network continuity. This combination can generate a hydrogel that is locally stiff under small deformation but more prone to swelling-induced expansion and fragmentation at the macroscopic level. Conversely, the more restricted swelling of HAF, together with its better apparent handling integrity, suggests that the higher molecular weight of AF-12 contributes to a more continuous and cohesive network, despite its lower storage modulus. Taken together, the rheological, visual, water-loss, and swelling results show that hydrogel performance cannot be evaluated solely from G' values. Instead, the behavior of these Ca^{2+} -crosslinked alginate hydrogels reflects a balance between G-block-mediated crosslinking, molecular weight, chain entanglement, and network continuity. HAE represents a system with high small-amplitude stiffness but lower macroscopic integrity, whereas HAF shows lower stiffness but more favorable apparent cohesion and more restricted swelling. Therefore, the extraction route indirectly governs hydrogel behavior by modifying both the block architecture and chain-length characteristics of the recovered alginate.

3.3. NMR Analysis of Alginate Structure

Since the rheological behavior of alginate is strongly influenced by monomer composition and sequence distribution, ^1H NMR analysis was used to examine how the extraction route affected the microstructure of the recovered polysaccharides. Particular attention was given to the relative abundance of guluronic and mannuronic residues, as well as to dyad and triad block distributions, because these parameters are directly related to ionic crosslinking capacity and gel-forming behavior. Figure 6 shows the ^1H NMR spectra of sodium alginates AE-5, AF-12 and CSA. All spectra exhibit well-resolved signals in the anomeric region, enabling reliable identification and quantification of guluronic acid (G) and mannuronic acid (M) units, as well as their sequence distributions along the polymer chain. Representative ^1H NMR spectra supporting the quantitative analysis of mannuronic (M) and guluronic (G) residues and block distribution are provided in the Supplementary Information (Figures S14–S16).

Peak assignments were performed in accordance with established literature and ASTM F2259-10 guidelines (see Section 2) [86–88]. In the anomeric region (Figure 6), the characteristic signals include the anomeric proton of guluronic acid (G-1) at 5.06 ppm (peak A), the H-5 proton of guluronic acid (G-5) at 4.45 ppm (peak C), and the anomeric proton of mannuronic acid (M-1) at 4.66 ppm (peak B_4). Additional signals corresponding to guluronic and mannuronic residues in mixed environments (signals B_1 – B_3) were also observed [89]. Overlapping resonances were resolved by peak deconvolution, allowing accurate integration of individual components.

These integrated peak areas were used to calculate the chemical composition of the alginate samples following the established methodology of Grasdalen et al. [89] this quantitative approach is based on the relative integrals of the characteristic ^1H NMR signals A, B_1 , B_2 , B_3 , B_4 , and C assigned in the anomeric region. The corresponding relationships used to determine monomer fractions, dyad and triad block distributions are given below. The molar fractions of guluronic (G) and mannuronic (M) units were calculated as:

$$G = 0.5(A + C + 0.5(B_1 + B_2 + B_3)) \quad (5)$$

$$M = B_4 + 0.5(B_1 + B_2 + B_3) \quad (6)$$

$$GG = 0.5(A + C - 0.5(B_1 + B_2 + B_3)) \quad (7)$$

$$MG = GM = 0.5(B_1 + B_2 + B_3) \quad (8)$$

$$MM = B_4 \quad (9)$$

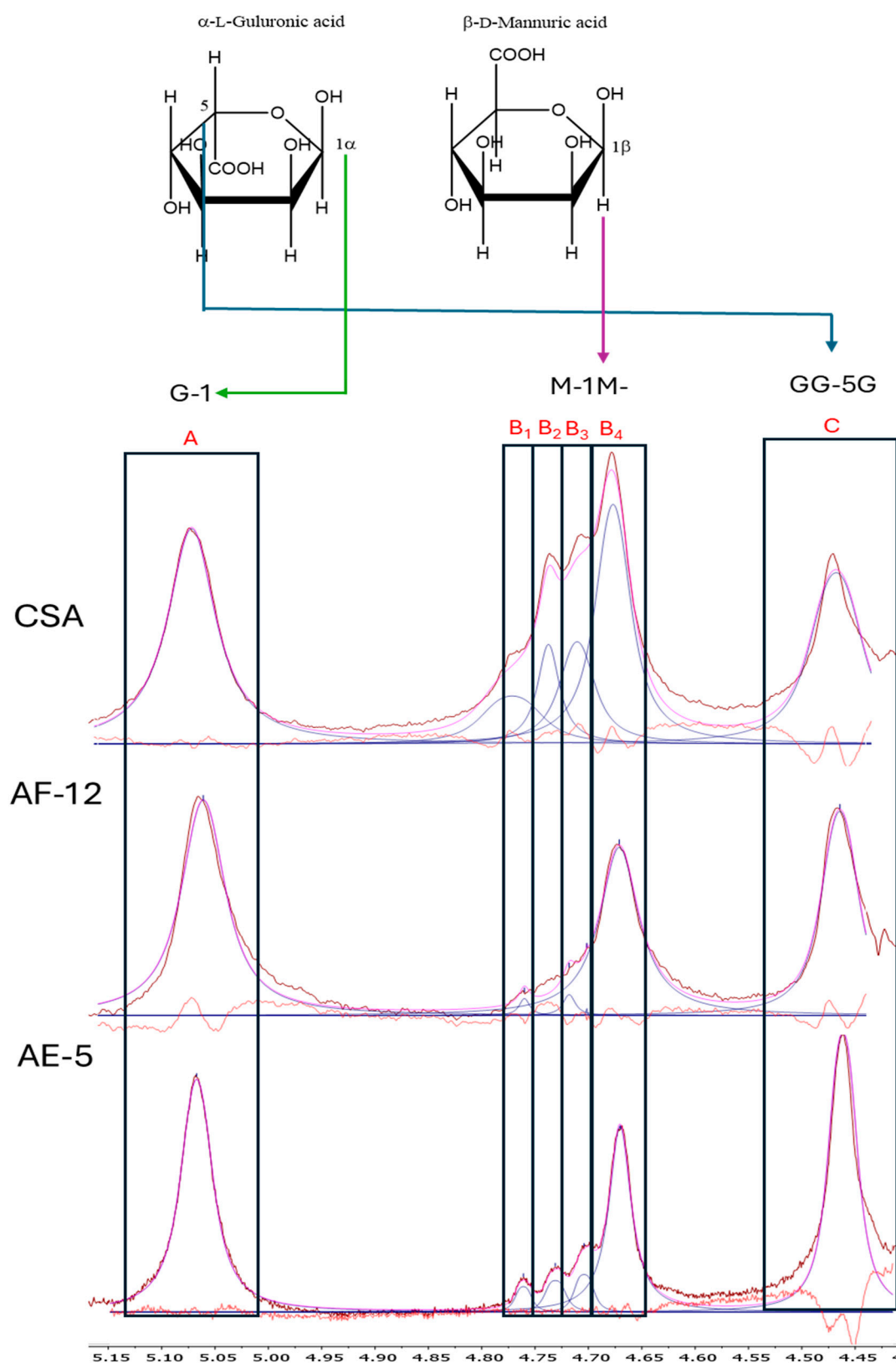


Figure 6. ^1H NMR spectra of sodium alginates: Comparison between CSA, AF-12, and AE-5.

Triad block fractions were calculated using:

$$\text{GGM} = \text{MGG} = (B_1)0.5(B_1 + B_2 + B_3)/(B_1 + B_2) \quad (10)$$

$$\text{MGM} = (B_2)0.5(B_1 + B_2 + B_3)/(B_1 + B_2) \quad (11)$$

$$GGG = GG - GGM \quad (12)$$

Normalized block fractions were then obtained as:

$$F_G = G/(M + G) \quad (13)$$

$$F_M = M/(M + G) \quad (14)$$

$$F_{\underline{GG}} = GG/(M + G) \quad (15)$$

$$F_{\underline{MM}} = MM/(M + G) \quad (16)$$

$$F_{\underline{GM}} = F_{\underline{MG}} = MG/(M + G) \quad (17)$$

$$F_{\underline{GGG}} = GGG/(M + G) \quad (18)$$

$$F_{\underline{MGM}} = MGM/(M + G) \quad (19)$$

$$F_{\underline{GGM}} = F_{\underline{MGG}} = GGM/(M + G) \quad (20)$$

Finally, the mannuronic-to-guluronic ratio (M/G) was calculated as:

$$M/G = (1 - F_G)/F_G \quad (21)$$

This analysis allows quantitative determination of monomer composition, block distribution, and sequential microstructure of the alginate chains. The resulting parameters (F_G , F_M , F_{GG} , F_{MM} , F_{MG} , and M/G) were used to compare alginates extracted via the formaldehyde (AF-12) and ethanol (AE-5) routes with literature data for other brown algae species, as discussed below.

AF-12 exhibited F_G and F_M values of 0.55 and 0.45, respectively, corresponding to an M/G ratio of 0.82. These values place AF-12 within the compositional range reported for several *Sargassum* species. For instance, although its overall composition is comparable to that of *S. turbinarioides* ($F_G = 0.52$, $F_M = 0.48$, $M/G = 0.94$), AF-12 displays a slightly higher guluronic acid content. Notably, the F_{GG} value of AF-12 of 0.53 exceeds that reported for *S. turbinarioides* (0.39), indicating a higher abundance of consecutive G residues, which are known to form cooperative binding sites for divalent cations during gelation [41].

AE-5 showed a higher guluronic acid content ($F_G = 0.60$, $F_M = 0.40$, and $M/G = 0.67$). Compared to *S. vulgare* and *S. asperifolium* (both with $F_G = 0.59$, $F_M = 0.41$, $M/G \approx 0.70$), AE-5 maintains a higher proportion of G residues while preserving a balanced block distribution. Its F_{GG} value (0.54) surpasses those reported for *S. vulgare* (0.49), *S. asperifolium* (0.48) and the commercial reference (0.27), indicating effective preservation of homopolymeric G-blocks during extraction.

In contrast, alginates from species such as *S. muticum* ($F_G = 0.76$, $F_M = 0.24$, $M/G = 0.31$) and *S. polycystum* ($F_G = 0.82$, $F_M = 0.18$, $M/G = 0.21$) exhibit guluronic acid dominance [90]. Although such compositions are known to yield highly rigid gels, they often compromise flexibility and processability. In this context, the intermediate compositions observed for AF-12 and AE-5 may be relevant for future material-oriented formulations, pending application-specific validation. As summarized in Table 6, their homopolymeric block fractions further highlight these differences, with AF-12 exhibiting $F_{MM} = 0.43$ and $F_{GG} = 0.53$, and AE-5 showing $F_{MM} = 0.34$ and $F_{GG} = 0.54$.

Table 6. Compositional information of sodium alginates extracted from brown algae species.

Species	F_G	F_M	F_{GG}	F_{MM}	F_{MG}	M/G	Reference
<i>Laminaria digitata</i>	0.47	0.53	0.41	0.47	0.06	1.12	Fertah et al. (2017) [41]
<i>Sargassum natans</i>	0.68	0.32	0.61	0.25	0.07	0.47	Rhein-Knudsen et al. (2017) [15]
<i>Sargassum vulgare</i>	0.59	0.41	0.49	0.31	0.10	0.70	
<i>Padina gymnospora</i>	0.36	0.64	0.21	0.48	0.16	1.75	
<i>Padina antillarum</i>	0.35	0.65	0.15	0.45	0.20	1.85	
<i>Laminaria digitata</i>	0.31	0.69	0.16	0.53	0.16	2.19	
<i>Macrocystis pyrifera</i>	0.34	0.66	0.13	0.45	0.21	1.94	
<i>Sargassum turbinarioides</i> <i>Grunow</i>	0.52	0.48	0.39	0.36	0.25	0.94	Fenoradosoa et al. (2010) [27]
<i>Sargassum fluitans</i>	0.64	0.36	0.55	0.28	0.08	0.57	Davis et al. (2004) [38]
<i>Saccharina longicruris</i>	0.59	0.41	0.25	0.07	0.34	0.69	Rioux et al. (2007) [86]
<i>Ascophyllum nodosum</i>	0.54	0.46	0.36	0.28	0.18	0.85	
<i>Fucus vesiculosus</i>	0.41	0.59	0.22	0.39	0.19	1.44	
<i>Sargassum asperifolium</i>	0.59	0.41	0.48	0.30	0.11	0.69	Larsen et al. (2003) [87]
<i>Sargassum filipendula</i>	0.56	0.44	0.45	0.33	0.11	0.78	Bertagnolli et al. (2014) [88]
<i>Sargassum filipendula</i>	0.84	0.16	0.76	0.07	0.08	0.19	Zhang et al. (2021) [91]
<i>Sargassum muticum</i>	0.76	0.24	0.59	0.07	0.17	0.31	
<i>Sargassum oligocystum</i>	0.57	0.43	0.37	0.24	0.20	0.77	
<i>Sargassum polycystum</i>	0.82	0.18	0.77	0.12	0.05	0.21	
<i>Sargassum thunbergii</i>	0.80	0.20	0.75	0.16	0.05	0.25	
<i>Sargassum</i> spp. (AF-12)	0.55	0.45	0.53	0.43	0.02	0.82	
<i>Sargassum</i> spp. (AE-5)	0.60	0.40	0.54	0.34	0.05	0.67	This study
Commercial sodium alginate	0.47	0.53	0.27	0.34	0.19	1.14	

The low F_{MG} values observed for both AF-12 (0.02) and AE-5 (0.05), relative to species such as *Sargassum oligocystum* (0.20) or *Ascophyllum nodosum* (0.18), confirm a predominantly blockwise architecture with limited alternating sequences. This structural feature is consistent with the formation of ordered junction zones upon ionic crosslinking. Triad sequence analysis (Table 7) further supports these observations. Both AF-12 and AE-5 exhibit high F_{GGG} values (0.52 and 0.51, respectively), confirming the dominance of extended G-block sequences. The negligible F_{MGM} and F_{GGM} fractions observed for AF-12 (0.0009 and 0.003) and AE-5 (0.003 and 0.022) indicate a low prevalence of mixed triads, reinforcing the block-like character of the polymer chains. Such triad distributions are known to favor the formation of rigid, ionically crosslinked networks. Taken together, the NMR results show that both extraction routes preserve a predominantly blockwise alginate architecture, but with clear differences in guluronic acid content and block distribution. These route-dependent structural variations provide a molecular basis for the distinct viscoelastic responses observed in the corresponding Ca^{2+} -crosslinked hydrogels.

Table 7. Triad block fractions (F_{GGG} , F_{MGM} , and F_{GGM}) of sodium alginates.

Species	F_{GGG}	F_{MGM}	F_{GGM}	Reference
<i>Sargassum natans</i>	0.58	0.04	0.03	Rhein-Knudsen et al. (2017) [15]
<i>Sargassum vulgare</i>	0.44	0.05	0.05	
<i>Padina gymnospora</i>	0.17	0.12	0.03	
<i>Padina antillarum</i>	0.12	0.17	0.03	
<i>Laminaria digitata</i>	0.11	0.11	0.05	
<i>Macrocystis pyrifera</i>	0.10	0.17	0.03	
<i>Sargassum</i> spp. (AF-12)	0.52	0.0009	0.003	This study
<i>Sargassum</i> spp. (AE-5)	0.51	0.003	0.022	
Commercial sodium alginate	0.14	0.026	0.003	

3.4. Fourier Transform Infrared (FTIR) Spectroscopy

Figure 7 shows the FTIR spectra of CSA, AE-5, and AF-12. All spectra exhibit the characteristic absorption bands associated with alginate polysaccharides, confirming that the extraction procedures preserve the chemical identity of the polymer. Representative FTIR spectra of sodium alginates extracted by ethanol and formaldehyde, together with a commercial reference, are provided in the Supplementary Information (Figures S2–S13).

A broad absorption band centered at 3250 cm^{-1} is observed in all samples and is assigned to O-H stretching vibrations associated with extensive hydrogen bonding, in agreement with previous reports [29,61,92]. A weak band at 2919 cm^{-1} corresponds to C-H stretching vibrations of the polysaccharide backbone, as reported by Laroche and Michaud [60] and Fenoradosoa et al. [27].

In the fingerprint region, an intense band at 1593 cm^{-1} is assigned to the asymmetric stretching vibration of the carboxylate group (O–C–O), while the band at 1405 cm^{-1} corresponds to its symmetric stretching mode. These bands are characteristic of sodium alginate and are consistent with values reported by Lawrie et al. [93], (1596 and 1412 cm^{-1} , respectively). The presence of these carboxylate bands, together with the absence of an absorption band near 1730 cm^{-1} , confirms that the alginate is present in its sodium salt form rather than as alginic acid [61].

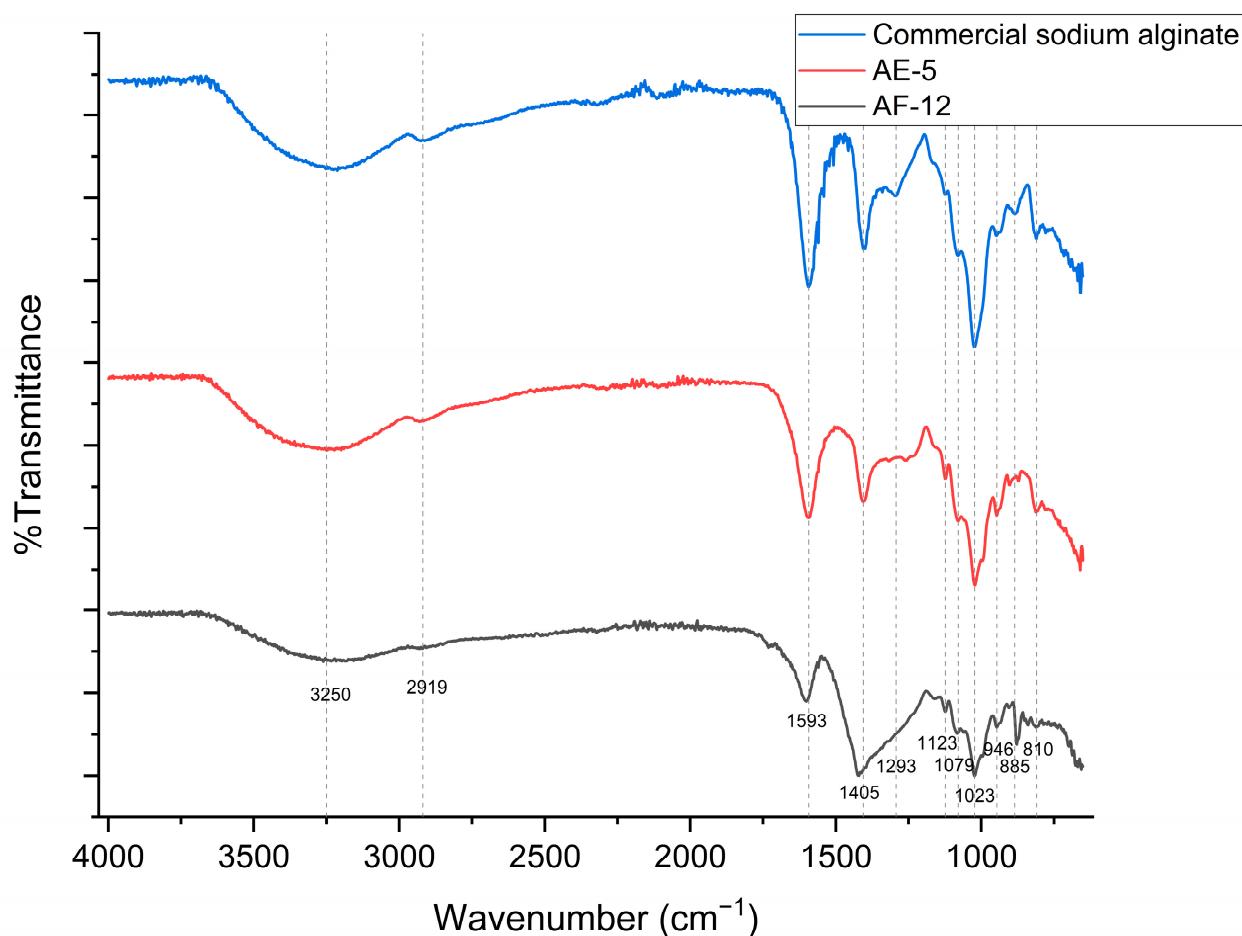


Figure 7. FTIR spectra of commercial sodium alginate (CSA), ethanol-derived sodium alginate (AE-5), and formaldehyde-derived sodium alginate (AF-12).

Additional bands observed at 1293 and 1123 cm^{-1} are attributed to C-C-H and O-C-H deformations and C-O stretching vibrations, respectively, in agreement with assignments reported by Khajouei et al. [29] Signals in the 1079–1023 cm^{-1} region correspond to C-O and C-C stretching vibrations of the pyranose rings, consistent with previous studies [41].

In the anomeric region (950–750 cm^{-1}), which is particularly informative for carbohydrate characterization [94], several diagnostic bands are observed. The band at 946 cm^{-1} is associated with C-O stretching vibrations of uronic acid residues [27], while the band at 885 cm^{-1} is attributed to the C1-H deformation vibration of β -mannuronic acid units [95]. The band at 810 cm^{-1} is characteristic of mannuronic acid residues, as reported in several studies [29,41,60,94].

The close similarity between the spectra of AE-5, AF-12, and the commercial reference supports the identification of sodium alginate as the predominant polysaccharide in the extracted samples and indicates that the extraction procedures did not introduce substantial chemical changes in the main functional groups of the polymer [94].

Although FTIR confirms the preservation of the characteristic functional groups of sodium alginate, this analysis does not provide a complete assessment of sample purity or residual contaminants. Therefore, the spectral similarity between the extracted alginates and the commercial reference should be interpreted as evidence of alginate formation and preservation of the main polysaccharide structure, rather than as proof of application-grade purity. Considering the heterogeneous origin of beach-cast *Sargassum*, a preliminary inorganic residue screening and selected metal analysis is included and discussed in the following section.

3.5. Preliminary Inorganic Residue Screening and Selected Metal Analysis

Considering the beach-cast origin of the *Sargassum* biomass, a preliminary assessment of inorganic residues and selected metal contaminants was incorporated into the revised manuscript. X-ray diffraction (XRD) was used as a qualitative screening tool to examine the possible presence of crystalline residual inorganic phases in the extracted alginates. The corresponding XRD patterns are provided in the Supporting Information File (Figure S19), since this analysis was intended only as a preliminary qualitative evaluation and not as a quantitative purity assessment. Briefly, the commercial sodium alginate showed the expected predominantly amorphous profile, whereas the alginates extracted from beach-cast *Sargassum* displayed weak crystalline reflections superimposed on the amorphous polymer background. These signals suggest the possible presence of residual crystalline inorganic phases, such as salts or mineral residues, although unambiguous phase assignment was not attempted because of the complexity of the polysaccharide matrix and the overlapping nature of weak diffraction signals.

To complement this qualitative screening, selected metal analysis was performed by microwave plasma atomic emission spectroscopy (MP-AES) after acid digestion. The analyzed elements were As, Cd, Cu, and Pb, which were selected because of their relevance in beach-cast marine biomass and environmental contamination assessment. The results are summarized in Table 8. Copper was detected in both extracted alginates, with concentrations of 5.8 mg kg^{−1} for AE-5 and 4.0 mg kg^{−1} for AF-12. Arsenic was not detected in AE-5 but was detected in AF-12 under the analytical conditions employed. Cadmium and lead were not detected in either sample.

Table 8. Selected metal analysis of *Sargassum*-derived alginates determined by MP-AES after acid digestion.

Sample	As (mg kg ^{−1})	Cd (mg kg ^{−1})	Pb (mg kg ^{−1})	Cu (mg kg ^{−1})
AE-5	ND	ND	ND	5.8
AF-12	DNQ	ND	ND	4.0

Note: Values are expressed as mg kg^{−1} of dry alginate. ND = not detected under the analytical conditions employed. DNQ = detected, not quantified. For As in AF-12, a weak signal was observed but could not be reliably quantified because it was below the quantification capability of the MP-AES method under the sample mass and dilution conditions used.

These results should be interpreted as a preliminary selected-metal screening rather than a complete contaminant or safety assessment. In particular, the DNQ result for As in AF-12 indicates that arsenic-related concerns cannot be fully resolved by the present MP-AES analysis. Accurate quantification and speciation would require more sensitive methods. Therefore, the extracted alginates are considered research-grade materials pending further purification and contaminant evaluation.

3.6. Structure-Property Relationships and Application-Oriented Limitations

Having established the extraction performance, molecular structure, rheological behavior, hydrogel integrity, swelling response, functional group preservation, and preliminary contaminant profile, the combined results reveal a route-dependent structure-property relationship governing the behavior of the recovered alginates. The ethanol-based route afforded the highest extraction yield and produced alginate enriched in guluronic acid and G-rich blocks. This composition favored the formation of Ca²⁺-crosslinked networks with the highest storage modulus under small-amplitude oscillatory deformation. Accordingly, HAE showed the highest G' value at 1 Hz and the highest apparent small-amplitude elastic stiffness estimated from rheological data. These findings indicate that the ethanol-derived alginate promotes efficient local Ca²⁺-mediated junction zone formation, consistent with

its higher guluronic acid content and G-block fraction. However, hydrogel performance was not determined by G-block content or stiffness alone. AE-5 also exhibited the lowest viscosity-average molecular weight, which appears to limit long-range chain connectivity and network continuity. This explains why HAE, despite showing the highest small-amplitude stiffness, displayed visible fragmentation during handling and higher long-term swelling. These results indicate that the high local crosslinking capacity of AE-5 does not fully translate into superior macroscopic integrity. Instead, the combination of short chain length and dense local junction zones may favor the formation of stiff but less cohesive hydrogel domains.

In contrast, AF-12 showed lower extraction yield and slightly lower guluronic acid content than AE-5, but it exhibited the highest intrinsic viscosity and viscosity-average molecular weight. This higher chain length appears to favor intermolecular entanglement and more continuous network formation. Consequently, HAF showed lower G' than HAE but better apparent macroscopic integrity during handling and a more restricted swelling profile. These observations support the interpretation that AF-12 provides a more balanced material profile, where moderate Ca^{2+} -mediated crosslinking is combined with improved long-range network connectivity.

Therefore, AE-5 and AF-12 should not be interpreted as universally superior or inferior materials, but as alginates with different property balances. AE-5 combines higher extraction yield, higher G-block content, and higher small-amplitude stiffness, but its lower molecular weight compromises macroscopic cohesion and favors greater swelling. AF-12, in contrast, provides lower yield and lower small-amplitude stiffness, but higher molecular weight, better apparent network continuity, and more controlled swelling behavior. These differences demonstrate that extraction route acts as an upstream variable that defines the balance between recovery efficiency, molecular preservation, block architecture, viscoelastic response, and macroscopic hydrogel behavior.

The preliminary contaminant-related analyses further reinforce that material selection cannot be based only on yield and rheological performance. XRD screening suggested the presence of residual crystalline inorganic phases in the extracted alginates and selected-metal analysis by MP-AES showed detectable Cu in both samples, while As was detected only in the formaldehyde-derived alginate. Although these measurements provide useful preliminary information, they do not constitute a complete purity or safety assessment. Thus, the extracted alginates should be considered structurally and rheologically characterized research-grade materials rather than application-ready products. Future application-specific use will require expanded contaminant profiling, arsenic speciation, purification optimization, biological evaluation, and direct mechanical testing under relevant use conditions.

The results show that the ethanol route favors yield and small-scale stiffness, whereas the formaldehyde route better preserves chain length and apparent hydrogel cohesion. This distinction provides a practical framework for selecting extraction conditions according to the desired balance between polysaccharide recovery, molecular structure, and hydrogel performance, while clearly defining the additional purification and validation steps required before any food, biomedical, pharmaceutical, or environmental application can be proposed.

4. Conclusions

Sodium alginate was successfully extracted from beach-cast *Sargassum* spp. collected on the Yucatán coast using two established pretreatment routes based on formaldehyde and ethanol. The results demonstrate that the extraction route controls not only alginate recovery, but also the molecular and supramolecular features that determine hydrogel

behavior. The ethanol-based route afforded the highest extraction yield, with AE-5 reaching $19.87 \pm 0.79\%$ *w/w*, whereas the formaldehyde-based route reached a maximum yield of $15.60 \pm 0.62\%$ *w/w* for AF-12. Statistical analysis confirmed that extraction conditions significantly affected alginate recovery, supporting the selection of AE-5 and AF-12 as representative high-yielding samples from each route. Despite its lower extraction yield, AF-12 exhibited higher intrinsic viscosity and viscosity-average molecular weight than AE-5, indicating better preservation of polymer chain length during the formaldehyde-based extraction route. In contrast, AE-5 showed higher guluronic acid content, lower *M/G* ratio, and a higher G-block fraction, as determined by ^1H NMR. These structural differences directly influenced hydrogel formation. AE-5-derived hydrogels displayed the highest storage modulus under small-amplitude oscillatory deformation, consistent with enhanced Ca^{2+} -mediated junction zone formation promoted by G-rich block sequences. However, the additional visual inspection, water-loss, and swelling analyses incorporated in the revised manuscript showed that higher small-amplitude stiffness did not translate into superior macroscopic integrity. HAE displayed visible fragmentation during handling and higher long-term swelling, which is consistent with reduced long-range network connectivity associated with its lower molecular weight. In contrast, AF-12-derived hydrogels exhibited lower storage modulus but better apparent macroscopic cohesion and a more restricted swelling profile. This behavior suggests that the higher molecular weight of AF-12 favors chain entanglement and network continuity, partially compensating for its lower stiffness. Therefore, the comparative analysis demonstrates that hydrogel performance is governed by a balance between G-block-mediated crosslinking, polymer chain length, network connectivity, swelling response, and handling integrity, rather than by extraction yield or storage modulus alone.

FTIR analysis confirmed the preservation of the characteristic functional groups of sodium alginate in the extracted samples, supporting the recovery of alginate as the predominant polysaccharide fraction. In addition, preliminary XRD screening, included in the SI File, suggested the presence of residual crystalline inorganic phases in the extracted alginates. To further address contaminant-related concerns associated with beach-cast biomass, selected-metal analysis was performed by MP-AES after acid digestion. Copper was quantified in both extracted alginates, whereas arsenic was detected but not quantified only in AF-12. Cadmium and lead were not detected under the analytical conditions employed. These results provide an initial inorganic-residue and selected-metal profile, but they do not constitute a complete purity or safety assessment if specific applications are desired. This study establishes a route-dependent structure-property relationship for sodium alginate extracted from beach-cast *Sargassum* spp. The ethanol route favors higher recovery, higher G-block content, and greater small-amplitude stiffness, whereas the formaldehyde route better preserves chain length and produces hydrogels with improved apparent cohesion and more restricted swelling. These findings support beach-cast *Sargassum* from the Yucatán coast as a promising source of research-grade sodium alginate and provide physicochemical, structural, rheological, and preliminary contaminant-related criteria for selecting extraction conditions according to the intended material requirements. Importantly, the extracted alginates should not be considered application-ready materials for food, biomedical, pharmaceutical, or environmental use at this stage. Future work should include purification optimization, expanded contaminant profiling, arsenic quantification and speciation, microbiological and biological evaluation, and direct macroscopic mechanical testing, including compression, fracture, and dimensional stability assays under application-relevant conditions. These additional studies will be necessary to validate the safety and performance of the extracted alginates for specific end-use applications.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/polysaccharides7030074/s1>. Figure S1: Strain sweep profiles of hydrogels HAF, HAE, and HAS showing the evolution of storage modulus (G') and loss modulus (G'') as a function of complex shear strain (%). Measurements were performed at a constant frequency of 1 Hz and 25 °C to determine the linear viscoelastic region (LVR), Figure S2: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-1), Formaldehyde (AF-1), and Commercial sodium alginate, Figure S3: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-2), Formaldehyde (AF-2), and Commercial sodium alginate, Figure S4: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-3), Formaldehyde (AF-3), and Commercial sodium alginate, Figure S5: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-4), Formaldehyde (AF-4), and Commercial sodium alginate, Figure S6: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-5), Formaldehyde (AF-5), and Commercial sodium alginate, Figure S7: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-6), Formaldehyde (AF-6), and Commercial sodium alginate, Figure S8: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-7), Formaldehyde (AF-7), and Commercial sodium alginate, Figure S9: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-8), Formaldehyde (AF-8), and Commercial sodium alginate, Figure S10: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-9), Formaldehyde (AF-9), and Commercial sodium alginate, Figure S11: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-10), Formaldehyde (AF-10), and Commercial sodium alginate, Figure S12: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-11), Formaldehyde (AF-11), and Commercial sodium alginate, Figure S13: FTIR Spectra of Sodium Alginates Extracted by Ethanol (AE-12), Formaldehyde (AF-12), and Commercial sodium alginate, Figure S14: Proton nuclear magnetic resonance spectra used to evaluate the composition and block distribution of mannuronic (M) and guluronic (G) acid residues in commercial sodium alginate, Figure S15: Proton nuclear magnetic resonance spectra used to evaluate the composition and block distribution of mannuronic (M) and guluronic (G) acid residues in AE-5, Figure S16: Proton nuclear magnetic resonance spectra used to evaluate the composition and block distribution of mannuronic (M) and guluronic (G) acid residues in AF-12, Figure S17: Representative photographs illustrating the accumulation of beach-cast Sargassum along the coastline of Progreso, Yucatán (Mexico), showing both large-scale shoreline coverage and close-up views of the stranded pelagic biomass used as raw material in this work, Figure S18: Photographs illustrating sodium alginate recovery during extraction from beach-cast Sargassum. (a) Alkaline extract after removal of insoluble residues. (b) Fibrous sodium alginate recovered after ethanol-induced precipitation and separation, Figure S19: X-ray diffraction (XRD) patterns of commercial sodium alginate (CSA), ethanol-derived alginate (AE-5), and formaldehyde-derived alginate (AF-12).

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Funding: External research funding was provided to M.A.F.-H. through Secihti Grant CF-2023-I-428.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Acknowledgments: L.F.J.-C. gratefully acknowledges the financial support from Secihti (formerly Conacyt) through PhD fellowship No. 924632. M.D.-F. acknowledges postdoctoral fellowship No. 473101. The authors are indebted to LANNBIO at Cinvestav Mérida and the Chemistry Department at Cinvestav Zacatenco for providing access to their research facilities. We extend our sincere appreciation to Ma. Teresa Cortez Picasso for her valuable assistance in NMR spectral acquisition. We also thank M.C. Ana Paola Montserrat Hernández Muñoz for acquiring the X-ray diffraction patterns,

and M.C. Mario Herrera and I.S.C. Georgina Espinoza for their technical support with the XRD analysis. During the preparation of this manuscript, the authors used ChatGPT (OpenAI, GPT-5.3) for the purposes of generating and refining the Graphical Abstract. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of 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. The authors declare no conflicts of interest.

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