

Cellulose Microfiber Production from Green Seaweed *Ulva lactuca* Using Hydrated Deep Eutectic Solvent

Rizfi Fariz Pari

pari.rizfi.784@es.kyushu-u.ac.jp

Kyushu University - Ito Campus: Kyushu Daigaku <https://orcid.org/0000-0003-4819-3588>

Safrina Dyah Hardiningtyas

IPB University: Institut Pertanian Bogor

Wahyu Ramadhan

IPB University: Institut Pertanian Bogor

Uju Uju

IPB University: Institut Pertanian Bogor

Rie Wakabayashi

Kyushu University: Kyushu Daigaku

Masahiro Goto

Kyushu University: Kyushu Daigaku

Noriho Kamiya

Kyushu University: Kyushu Daigaku <https://orcid.org/0000-0003-4898-6342>

Research Article

Keywords: cellulose microfiber, cellulose microparticle, hydrated deep eutectic solvent, pretreatment, seaweed biomass

Posted Date: June 2nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6731198/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Biotechnology Letters on February 5th, 2026. See the published version at <https://doi.org/10.1007/s10529-026-03702-y>.

Abstract

Purpose

We investigated the effectiveness of hydrated deep eutectic solvent (DES) treatments at modulating the morphology of *Ulva lactuca* cellulose to enable the selective production of seaweed cellulose microfibers (SCMF) with distinct functionality.

Methods *Ulva lactuca*

cellulose was extracted by sequential removal of water soluble material, lignin and pigment using hot water, NaOH and H₂O₂, respectively. The extracted cellulose was then treated by various hydrated DES (30% DES in water) in combination with homogenization and sonication, yielding SCMF.

Results

Treatment with 30% hydrated DES composed of choline chloride (ChCl) or betaine as hydrogen bond acceptors and urea, citric acid, or oxalic acid as hydrogen bond donors combined with homogenization and sonication successfully produced SCMF. The finest SCMF was obtained using ChCl:urea, yielding fibers with a dry diameter of 371.7 nm. The other DES treatments produced spherical seaweed cellulose microparticles with an average diameter between 605 and 777 nm. The SCMF exhibited exceptional dispersibility in water, with a hydrodynamic diameter of 134.94 nm and good homogeneity, with a polydispersity index of 0.23. Notably, only the ChCl:urea treatment produced a SCMF that remained predominantly amorphous, while all other hydrated DES treatments significantly increased crystallinity. Treatment with ChCl:oxalic acid introduced carboxyl groups into the structure.

Conclusion

These findings demonstrate tunable cellulose morphology, crystallinity and excellent water dispersibility through selecting appropriate hydrated DES combinations for potential application in sustainable material and functional material systems.

Introduction

Seaweed is an abundant and sustainable biomass resource with fast growth rates and high carbon capture potential (Ramachandra and Hebbale 2020). With particular promise, *Ulva lactuca* is a green seaweed with a high carbohydrate content, including ulvan, cellulose, and hemicellulose (Pari et al. 2024). While ulvan has diverse applications in food, cosmetics, and pharmaceuticals (Kidgell et al. 2019), its extraction leaves cellulose-rich residues that can be further valorized.

Cellulose microfibers (CMFs), especially those originating from amorphous cellulose, are highly valued for their light weight and biodegradability. CMFs can be used to improve cement paste quality (Gwon and Shin 2021), for drug delivery for poorly soluble drugs (Löbmann and Svagan 2017), as a cosmetic

ingredient (Jang et al. 2021), and as a supercapacitor (Fukuhara et al. 2021). The dispersibility properties of CMF are important to ensure their uniform distribution in food and biomedical application. Conventional CMF production methods rely on harsh chemicals such as sulfuric acid and 2,2,6,6-tetramethylpiperidine-1-oxylradical (TEMPO), which raise environmental concerns (Salem and Ismail 2022). Thus, interest in green solvents, such as deep eutectic solvents (DESs) as alternatives for biomass pretreatment, is growing.

DESs, often considered as low-cost, eco-friendly substitutes for ionic liquids (ILs), are composed of hydrogen bond acceptors (HBA) and donors (HBD) (Płotka-Wasyłka et al. 2020). A treatment with 100% DES consisting of choline chloride (ChCl) as the HBA with urea, citric acid and oxalic acid as the HBD together with a mechanical treatment such as microfluidization, ultrasonication and homogenization on wood cellulose and bagasse induced fibrillation (Sirviö et al. 2015; Li et al. 2021; Pradhan et al. 2024). Despite their potential, common DESs such as ChCl:urea, ChCl:oxalic acid exhibit high viscosity, which limits their processing efficiency. This can be mitigated by hydrating the DES with water, which lowers viscosity and improves cellulose processing performance (Amoroso et al. 2021). Adding water to DES will also reduce the cost, because less material is used. However, the application of DESs, including hydrated formulations, for treating *U. lactuca* cellulose remains underexplored.

Therefore, this study aimed to evaluate the effectiveness of various hydrated DES combinations, using choline chloride or betaine as the HBA and urea, oxalic acid, or citric acid as the HBD, to pretreat *U. lactuca* cellulose obtained as a by-product from ulvan extraction.

Materials and methods

Materials

The materials used in this study were NaOH (Merck KGaA., Darmstadt, Germany), H₂O₂, choline chloride (ChCl), and oxalic acid (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), citric acid, betaine, and urea (Kishida Chemical Co., Ltd., Osaka, Japan). *U. lactuca* was collected from Minajaya Beach, Sukabumi, West Java, Indonesia (-7 ° 23 ' 58.14 ", 106 ° 31 ' 0.61 ").

Cellulose extraction from *Ulva lactuca*

The *U. lactuca* was washed twice with seawater and distilled water then air-dried, and milled into a powder. The seaweed powder was stored in a vacuum-sealed plastic bag at room temperature. Cellulose was extracted from the seaweed by removing the water-soluble material, lignin and color. To remove the water-soluble material, the seaweed powder was heated and continuously stirred in distilled water at a ratio of 1:20 (w/v) at 90°C for 2 h. The sample material was filtered out using a 100-micron nylon mesh then dried at room temperature. For delignification, the sediment was boiled in 10% NaOH at a ratio of 1:20 (w/v) at 100°C for 2 h at 400 rpm. The sediment was filtered using a 100-micron nylon mesh, washed using distilled water until attaining a pH of 7 then air-dried at room temperature. For bleaching, the sediment was boiled in 6% H₂O₂ at a ratio of 1:20 (w/v) at 80°C for 3 h at 400 rpm. The precipitate

was filtered using a 100-micron nylon mesh, washed using distilled water until the pH value was 7 then air-dried at room temperature to obtain the raw seaweed cellulose (RSC).

U. lactuca cellulose deep eutectic solvent treatment

DESs with a combination of ChCl:urea (1:2), betaine:urea (1:3), ChCl:citric acid (1:1), ChCl:oxalic acid (1:1) and betaine:oxalic acid (1:2) were prepared in molar ratio. The materials were heated at 100°C until a clear, colorless liquid was obtained. The DES was diluted with distilled water to a concentration of 30% for the treatment experiments and also to 10%, 20%, and 70% for the ChCl:urea treatments. The RSC was treated by hydrated DES at a ratio of 1:20 (w /v) at 80°C for 1 h at 600 rpm. The sample was then centrifuged at 8,000 *g* and 20°C for 15 min then washed with distilled water. This step was repeated 3 times. The sample was added to distilled water and homogenized at 4000 rpm for 30 min (Polytron PT 3100 D, Kinematica AG, Lucerne, Switzerland), then sonicated (UD-201, Tomy Seiko Co. Ltd., Tokyo, Japan) at 50% power, and 1200 W for 20 min. For purification, the sample was dialyzed by double-distilled water in a dialysis bag (MWCO 12–14 kDa; Spectra/Por 2: Repligen, Waltham, MA, USA) for 3 d. The seaweed cellulose microfibers (SCMF) obtained were freeze-dried for further analysis. The SCMF yield was calculated based on the initial cellulose weight. Figure 1 illustrates the overall process for producing RSC and SCMF from *U. lactuca*.

Morphological analysis

The structures of RSC and SCMF were analyzed using Scanning Electron Microscopy (SEM) SU3500 (Hitachi High-Tech Corporation, Tokyo, Japan). The particle sizes were determined using ImageJ software.

Dispersion of SCMFs

The SCMFs were dispersed in distilled water at a ratio of 1:50 (w /v). The hydrodynamic diameter and polydispersity index (PDI) of the SCMF in water were characterized by dynamic light scattering (DLS) (NanoZSP, ZEN5600, Zetasizer Nano, Malvern Panalytical Ltd., Malvern, UK).

Functional group analysis

The functional groups of RSC and SCMFs were analyzed using Fourier Transform Infrared Spectroscopy (FTIR, Spectrum Two, PerkinElmer, Waltham, MA, USA). The functional group spectra were measured over a wave number range of 4000 – 500 cm⁻¹.

Crystallinity analysis

The crystal structure was analyzed using X-Ray Diffraction (XRD) (SmartLab SE, Rigaku Corporation, Tokyo, Japan). The RSC or SCMF samples were spread evenly on an Si crystal plate which was then attached to the analyzer holder. The tool was conditioned with a Cu-K α radiation beam mode at a voltage of 40 kV and a generator current of 30 mA. The analysis was carried out using 2 θ angle diffraction at an

initial angle of 10 ° to a final angle of 80 ° with a reading speed of 2 ° per min. The degree of crystallinity Index (Crl) was determined by the Segal method (Segal et al. 1959).

Results and discussion

Yields of RSC and SCMFs

The raw seaweed cellulose (RSC) obtained from 100 g of *U. lactuca* biomass was 7.6 ± 0.9 g at the start of extraction, therefore the overall yield was approximately 8%. This value is comparable to the theoretical value of *U. lactuca* from Indonesia, potentially containing approximately 10% cellulose, depending on the harvest season and geographic location (Pari et al. 2024). The RSC was treated with various combinations of 30% hydrated DESs (30% DES, 70% water) followed by mechanical treatment to produce SCMFs. The yield of SCMF is shown in Fig. 2a. The yields of SCMF varied significantly between the various hydrated DES treatments. The repeated washing and purification steps, before and after mechanical treatment, caused the loss of yield. This result was also similar to DES-treated wood cellulose (Sirviö et al. 2024). Hydrated DES containing oxalic acid as the HBD tended to have significantly lower yields (58%) than other treatment. Oxalic acid has potential ability for cellulose hydrolysis (Li et al. 2017), contributing to a reduction in SCMF yield. In contrast, higher yields of SCMF (69%) were obtained by a treatment using urea as the HBD where it was reported that urea reacted with cellulose to form gelation and dissolved cellulose (Cai and Zhang 2006).

Dispersity of SCMFs

The dispersed state of 2% SCMFs in water was analyzed by DLS. The SCMF were homogeneous in solution with an average PDI of 0.23 (Fig. 2b). The PDI value is a dimensionless index in the range from 0 (perfectly homogenous particle size sample) to 1 (highly polydisperse sample with heterogenous size populations). For cellulose of a micro particle size, a PDI of less than 0.7 is considered highly polydispersed (Ceaser and Chimphango 2021), so the particle size distribution in the present study was relatively good. Besides being homogeneous, the SCMF particles were also pure, and uniform based on their hydrodynamic size in solution which ranged from 134 to 471 nm, the smallest from ChCl:urea treatment and the largest from ChCl:oxalic acid treatment (Fig. 2c). A well-dispersed SCMF is important for industrial application because it can guarantee a uniform distribution and stable suspension, for example, during formulation in drug delivery platforms (ranging from injectable systems to advanced bioprinted constructs) and in food emulsion stabilizers (mayonnaise and ice cream) (Tofanica et al. 2024).

The changes of morphological characteristics

The SEM measurements on RSF and SCMFs allowed the visual analysis of their structural characteristics. The SEM images (Fig. 3) show the changes in the RSC structure after hydrated DES treatment, homogenization and sonication. Before the treatment, the RSC was of a macro size with an irregularly wrinkled and thick surface. The hydrated DES and mechanical treatment, particularly for the

ChCl:urea, betaine:urea, and ChCl:citric acid treatments, created large pores in the surface and thinned the sheets of cellulose. Urea makes cellulose soluble in water (Walters et al. 2020). Hydrated ChCl:urea promoted partial deconstruction of the cellulose structure by weakening hydrogen bonds, rather than achieving full dissolution. Increasing the temperature weakens hydrogen bonds, so that at temperatures over 55°C, the cellulose begin to expand and stretch (Cai and Zhang 2006). After the treatment with oxalic acid as the HBD, the cellulose was degraded into smaller fractions. Hydrolysis of cellulose occurs in the presence of oxalic acid (Henschen et al. 2019), resulting in its disintegration.

Based on the SEM images, we determined the fiber diameter and particle size of the SCMFs (Fig. 2b). Of all the treatments, only the ChCl:urea treatment produced microfibers (diameter 372 ± 156 nm), while the other treatments produced spherical cellulose microparticles with diameters from 605 ± 246 to 777 ± 321 nm. The hydrolysis mechanism of RSC with ChCl:urea can be explained as follows: the urea carbonyl group and the chloride anion interact with the cellulose hydroxyl groups, catalyzing the breakdown of hydrogen bonding networks in the cellulose fiber (Smirnov et al. 2020). After mechanical treatments, the hydrolyzed portion degrades, thus fibers were formed predominantly in the ChCl:urea treatment. Using DES composed of ChCl:urea, different hydration concentrations were also conducted at 10%, 20%, and 70% of DES in water (**Supplementary Fig. 1**). However, in 10% ChCl:urea, the RSC did not turn into fibers, but formed a pipe-like shape (10.36 μ m diameter). However, in the 20% ChCl:urea treatment, the RSC formed both fibers and sheets. In the 70% ChCl:urea treatment, the RSC was still present in large compact masses. The hydrated DES conditions improved cellulose swelling easing the penetration of solvent into the cellulose (Sirviö et al. 2022). Therefore, 30% was considered the best concentration for hydrated ChCl:urea to obtain SCMF from RSC, as it produced a fibrous morphology with a high yield.

Structural characteristics of CMFs by XRD and FTIR

The crystallinity of the samples was examined using XRD (**Fig. 4a**). RSC showed the crystallinity peaks (I_{200}) at 20°. The I_{200} of CMFs were shifted to approximately 21°. In addition, SCMF from hydrated DES with oxalic acid as the HBD also showed new peaks at 15.01°, 26.63°, and 30.23°, corresponding with I_{101} , I_{004} , and I_{040} , respectively. These results suggested the formation of cellulose I where hydrated DES hydrolyzed cellulose caused the removal of amorphous component, the shifted peak and thus an increase in the crystallinity index. Hydrated DES with oxalic acid as the HBD promoted the reorganization of the cellulose chain into more ordered structures.

The crystallinity indices of *U. lactuca* cellulose and SCMFs are shown in **Fig. 4b**. The RSC from *U. lactuca* cellulose had a crystallinity index of 13%. The crystallinity indices from the present study were lower than those of CMFs from hardwood (Willberg-Keyriläinen et al. 2018). The combination of hydrated DES with mechanical treatment improved the crystallinity index. The ChCl:urea treatment did not change the crystallinity index, while the other treatments significantly increased it. For ChCl:oxalic acid treatment, the crystallinity index of SCMF at 29% was twice that of the RSC crystallinity index (13%). This was in line with research on kraft pulp cellulose which increased 5% after treatment with 30% ChCl:oxalic acid (Ma et al. 2019). The crystallinity index indicates the percentage of the crystalline area in cellulose (Salem et

al. 2023), so a crystallinity index of 30% means that 70% of the cellulose is amorphous, making it more soluble and dispersible in aqueous media. Amorphous CMF would thus lead to drugs of poor solubility (Löbmann and Svagan 2017). The amphiphilic property of CMF has been reported to make it suitable in stabilizing oil-in-water emulsions (Gestranius et al. 2017).

Figure 4c shows the FTIR results. The RSC exhibited peaks related to sulphated polysaccharide ulvan at 1459, 1259, and 846 cm^{-1} for COO^- , $\text{S}=\text{O}$ and COS , respectively. The peaks related to ulvan disappeared in all SCMFs, indicating that the treatments also produced purer cellulose. The presence of a peak at 3700 – 3584 cm^{-1} indicated the O-H stretching group. In the case of amorphous cellulose, the peak shifted to a higher wavelength because of the breaking of intra- and inter-molecular hydrogen bonds. The presence of amorphous cellulose was also confirmed by a slight peak shift of 2909 cm^{-1} for the C-H stretching group. There was an increase in water absorption with the presence of the O-H group peak at a wavelength of 1629 cm^{-1} , indicating the affinity of SCMFs towards water. The peak at 1320 cm^{-1} for CH bending was stronger in CMF treated by hydrated DES with oxalic acid as the HBD which was associated with crystalline cellulose. This wavenumber is also known as the “crystallinity band”, which indicated that the decrease in intensity was reflecting a decrease in the degree of crystallinity of the sample, and vice versa (Salem et al. 2023). The C-O-C stretching peak appeared at a wavelength of 1061 cm^{-1} , which indicated an amorphous structure. The peak found at a wavelength of 1671 cm^{-1} for the $\text{C}=\text{O}$ group, indicated that SCMF treated by hydrated DES with oxalic acid as the HBD had possibly gained an additional carboxyl and hemiacetal group. Simultaneous esterification and hydrolysis of cellulose can potentially occur in the presence of oxalic acid at elevated temperatures (Henschen et al. 2019). The appearance of carboxyl peaks was also observed in nanocellulose derived from kraft pulp treated with hydrated CO in combination with ultrasonication (Ma et al. 2019), highlighting the possible structural functionality imparted by the hydrated DES system.

Conclusion

The use of hydrated deep eutectic solvents (DESs) for the pretreatment of *Ulva lactuca* cellulose has been successfully demonstrated, thus enabling the production of cellulose microfibers (CMFs) with tailored structural and functional properties. Of the DES combinations studied, $\text{ChCl}:\text{urea}$ (30% hydration) was most effective at producing well-dispersed, and highly water-dispersible CMFs of low polydispersity with a preserved amorphous structure, which is desirable for applications in drug delivery and food emulsion stabilization. Conversely, hydrated DESs containing oxalic acid significantly enhanced the crystallinity and introduced carboxyl groups into the CMF structure, thus expanding their potential for functional material applications. Overall, the present study has highlighted the versatility of hydrated DES formulations as green and tunable pretreatment systems for seaweed-derived cellulose, offering an environmentally-friendly approach to producing CMFs with application-specific properties.

Statement and Declarations

Acknowledgement

We thank Philip Creed, PhD, from Edanz (<https://jp.edanz.com/ac>) for editing a draft of this manuscript.

Funding

This work was supported by the Japan Society for the Promotion of Science (JSPS) (Grant No. JPJSBP120228101) and the Directorate General of Higher Education (DGHE), Ministry of Higher Education, Science and Technology, Republic of Indonesia (Grant No. 069.2/E4.4/KU/2024).

Competing Interests

The authors declare no competing interests

Ethical approval

This study did not involve animal or human and there was no ethical approval is required.

Author Contributions

Material preparation, data collection, and analysis were performed by Rizfi Fariz Pari. *Ulva lactuca* preparation was conducted by Safrina Dyah Hardiningtyas and Wahyu Ramadhan. Project administration and supervision were provided by Rie Wakabayashi, Noriho Kamiya, and Masahiro Goto. Funding was acquired by Masahiro Goto and Uju. The first draft of the manuscript was written by Rizfi Fariz Pari. All authors reviewed and approved the final manuscript.

Data Availability

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

References

1. Amoroso R, Hollmann F, Maccallini C (2021) Choline Chloride-Based DES as Solvents/Catalysts/Chemical Donors in Pharmaceutical Synthesis. *Molecules* 26:6286. <https://doi.org/10.3390/molecules26206286>
2. Cai J, Zhang L (2006) Unique Gelation Behavior of Cellulose in NaOH/Urea Aqueous Solution. *Biomacromolecules* 7:183–189. <https://doi.org/10.1021/bm0505585>
3. Ceaser R, Chimphango AFA (2021) Comparative analysis of physical and functional properties of cellulose nanofibers isolated from alkaline pre-treated wheat straw in optimized hydrochloric acid and enzymatic processes. *Int J of Biol Macromol* 171:331–342. <https://doi.org/10.1016/j.ijbiomac.2021.01.018>

4. Fukuhara M, Kuroda T, Hasegawa F, et al (2021) Amorphous cellulose nanofiber supercapacitors. Sci Rep 11:6436. <https://doi.org/10.1038/s41598-021-85901-3>
5. Gestranus M, Stenius P, Kontturi E, et al (2017) Phase behaviour and droplet size of oil-in-water Pickering emulsions stabilised with plant-derived nanocellulosic materials. Colloids Surf A Physicochem Eng Asp 519:60–70. <https://doi.org/10.1016/j.colsurfa.2016.04.025>
6. Gwon S, Shin M (2021) Rheological properties of cement pastes with cellulose microfibers. J Mater Res Technol 10:808–818. <https://doi.org/10.1016/j.jmrt.2020.12.067>
7. Henschen J, Li D, Ek M (2019) Preparation of cellulose nanomaterials via cellulose oxalates. Carbohydr Polym 213:208–216. <https://doi.org/10.1016/j.carbpol.2019.02.056>
8. Jang JH, So BR, Yeo HJ, et al (2021) Preparation of cellulose microfibril (CMF) from *Gelidium amansii* and feasibility of CMF as a cosmetic ingredient. Carbohydr Polym 257:117569. <https://doi.org/10.1016/j.carbpol.2020.117569>
9. Kidgell JT, Magnusson M, de Nys R, Glasson CRK (2019) Ulvan: A systematic review of extraction, composition and function. Algal Res 39:101422. <https://doi.org/10.1016/j.algal.2019.101422>
10. Li C, Huang C, Zhao Y, et al (2021) Effect of Choline-Based Deep Eutectic Solvent Pretreatment on the Structure of Cellulose and Lignin in Bagasse. Processes 9:384. <https://doi.org/10.3390/pr9020384>
11. Li D, Henschen J, Ek M (2017) Esterification and hydrolysis of cellulose using oxalic acid dihydrate in a solvent-free reaction suitable for preparation of surface-functionalised cellulose nanocrystals with high yield. Green Chem 19:5564–5567. <https://doi.org/10.1039/C7GC02489D>
12. Löbmann K, Svagan AJ (2017) Cellulose nanofibers as excipient for the delivery of poorly soluble drugs. Int J Pharm 533:285–297. <https://doi.org/10.1016/j.ijpharm.2017.09.064>
13. Ma Y, Xia Q, Liu Y, et al (2019) Production of Nanocellulose Using Hydrated Deep Eutectic Solvent Combined with Ultrasonic Treatment. ACS Omega 4:8539–8547. <https://doi.org/10.1021/acsomega.9b00519>
14. Pari RF, Uju, Wijayanta AT, et al (2024) Prospecting Ulva lactuca seaweed in Java Island, Indonesia, as a candidate resource for industrial applications. Fish Sci 90:795–808. <https://doi.org/10.1007/s12562-024-01799-6>
15. Płotka-Wasyłka J, de la Guardia M, Andruch V, Vilková M (2020) Deep eutectic solvents vs ionic liquids: Similarities and differences. Microchem J 159:105539. <https://doi.org/10.1016/j.microc.2020.105539>
16. Pradhan D, Jaiswal S, Tiwari BK, Jaiswal AK (2024) Choline chloride – oxalic acid dihydrate deep eutectic solvent pretreatment of Barley straw for production of cellulose nanofibers. Int J Biol Macromol 281:136213. <https://doi.org/10.1016/j.ijbiomac.2024.136213>
17. Ramachandra TV, Hebbale D (2020) Bioethanol from macroalgae: Prospects and challenges. Renew Sustain Energy Rev 117:109479. <https://doi.org/10.1016/j.rser.2019.109479>
18. Salem DMSA, Ismail MM (2022) Characterization of cellulose and cellulose nanofibers isolated from various seaweed species. Egypt J Aquat Res 48:307–313.

<https://doi.org/10.1016/j.ejar.2021.11.001>

19. Salem K, Kasera NK, Rahman MA, et al (2023) Comparison and assessment of methods for cellulose crystallinity determination. *Chem Soc Rev* 52:6417–6446.
<https://doi.org/10.1039/D2CS00569G>
20. Segal L, Creely JJ, Martin Jr AE, Conrad CM (1959) An Empirical Method for Estimating the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer. *Text Res J* 29:786–794.
<https://doi.org/10.1177/004051755902901003>
21. Sirviö JA, Haataja R, Kantola AM, et al (2022) Insights into the role of molar ratio and added water in the properties of choline chloride and urea-based eutectic mixtures and their cellulose swelling capacity. *Phys Chem Chem Phys* 24:28609–28620. <https://doi.org/10.1039/D2CP04119G>
22. Sirviö JA, Romakkaniemi I, Ahola J, et al (2024) Supramolecular interaction-driven delignification of lignocellulose. *Green Chem* 26:287–294. <https://doi.org/10.1039/D3GC03857B>
23. Sirviö JA, Visanko M, Liimatainen H (2015) Deep eutectic solvent system based on choline chloride-urea as a pre-treatment for nanofibrillation of wood cellulose. *Green Chem* 17:3401–3406.
<https://doi.org/10.1039/C5GC00398A>
24. Smirnov MA, Sokolova MP, Tolmachev DA, et al (2020) Green method for preparation of cellulose nanocrystals using deep eutectic solvent. *Cellulose* 27:4305–4317. <https://doi.org/10.1007/s10570-020-03100-1>
25. Tofanica B-M, Mikhailidi A, Samuil C, et al (2024) Advances in Cellulose-Based Hydrogels: Current Trends and Challenges. *Gels* 10:842. <https://doi.org/10.3390/gels10120842>
26. Walters MG, Mando AD, Reichert WM, et al (2020) The role of urea in the solubility of cellulose in aqueous quaternary ammonium hydroxide. *RSC Adv* 10:5919–5929.
<https://doi.org/10.1039/C9RA07989K>
27. Willberg-Keyriläinen P, Hiltunen J, Ropponen J (2018) Production of cellulose carbamate using urea-based deep eutectic solvents. *Cellulose* 25:195–204. <https://doi.org/10.1007/s10570-017-1465-9>

Figures

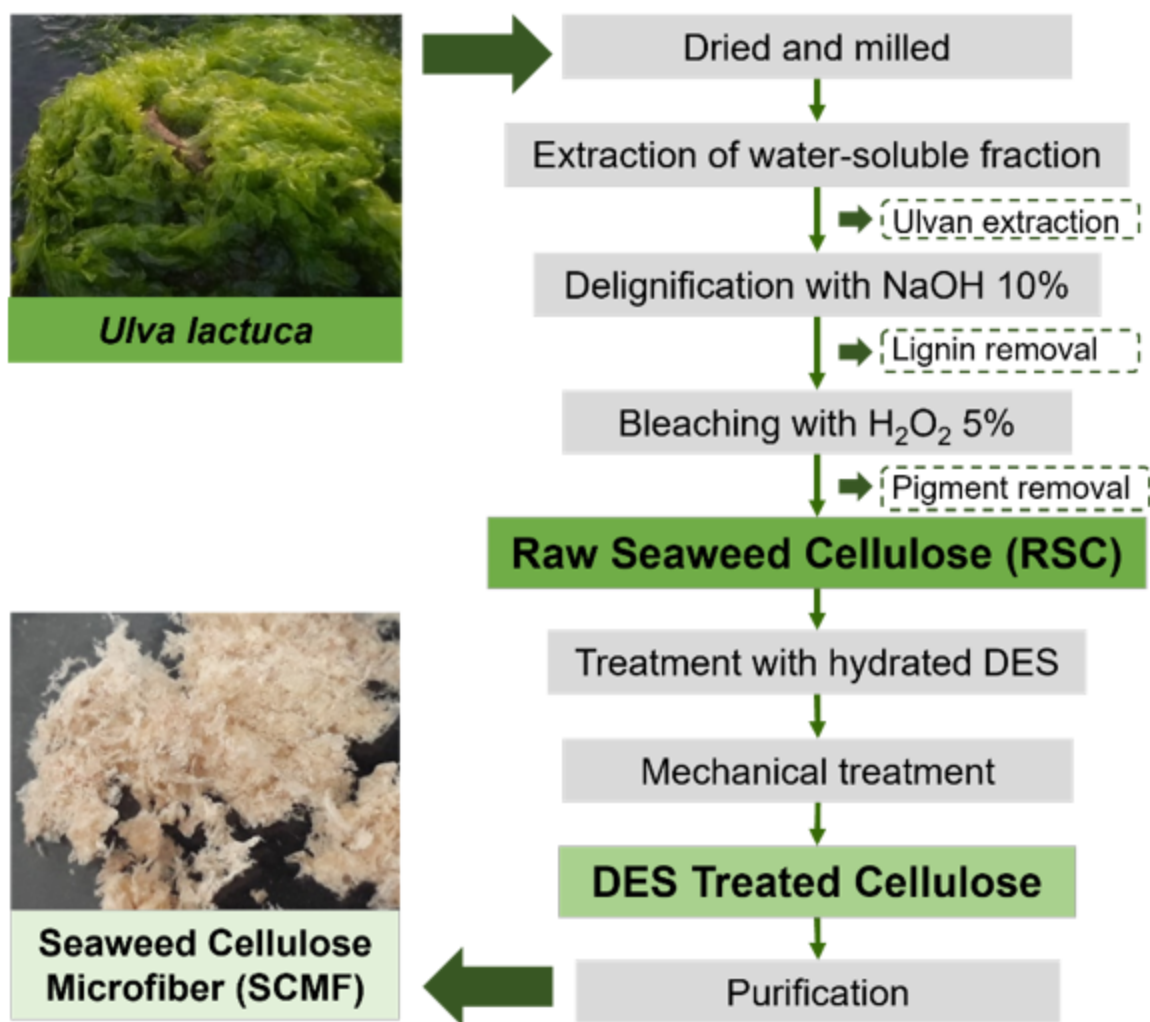


Figure 1

Seaweed cellulose microfiber (SCMF) production process from green seaweed *Ulva lactuca* using hydrated deep eutectic solvent (DES) treatment.

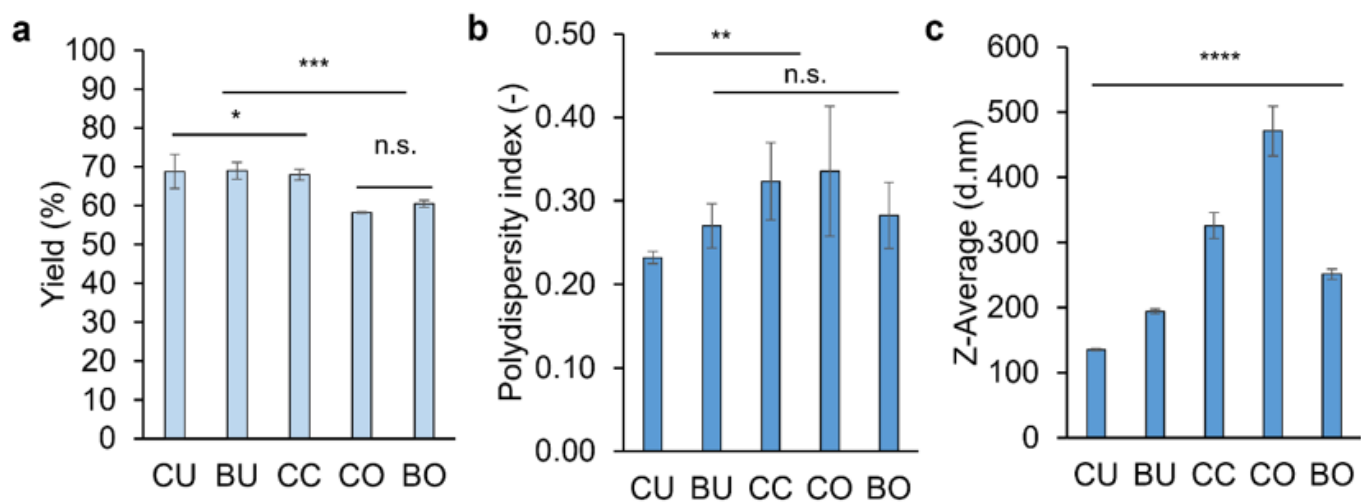


Figure 2

(a) The yield; (b) polydispersity index (PDI), and (c) Z-average hydrodynamic diameter of seaweed cellulose microfibril (SCMF) obtained after hydrated deep eutectic solvent (DES) treatments with 30% choline chloride:urea (CU), betaine:urea (BU), choline chloride: citric acid (CC), choline chloride:oxalic acid (CO), and betaine:oxalic acid (BO). Data are presented as mean $\pm$ SD (n = 3). Statistical analysis was performed using Tukey's multiple comparison test. Significant differences are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, and n.s. for not significant.

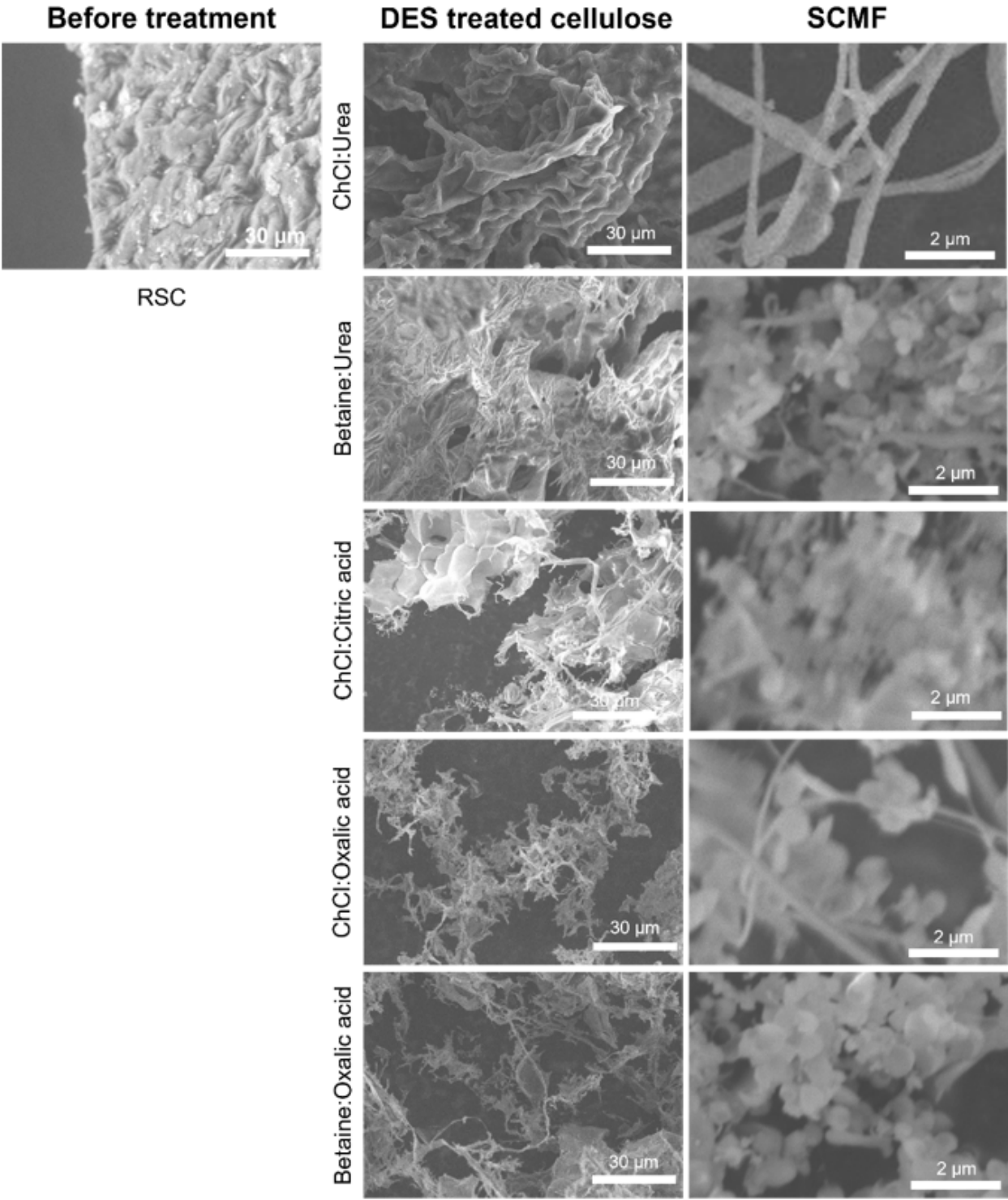


Figure 3

Scanning electron micrographs of raw seaweed cellulose (RSC) from *Ulva lactuca*, treated cellulose and seaweed cellulose microfibril (SCMF) produced using various hydrated deep eutectic solvent (DES) treatment.

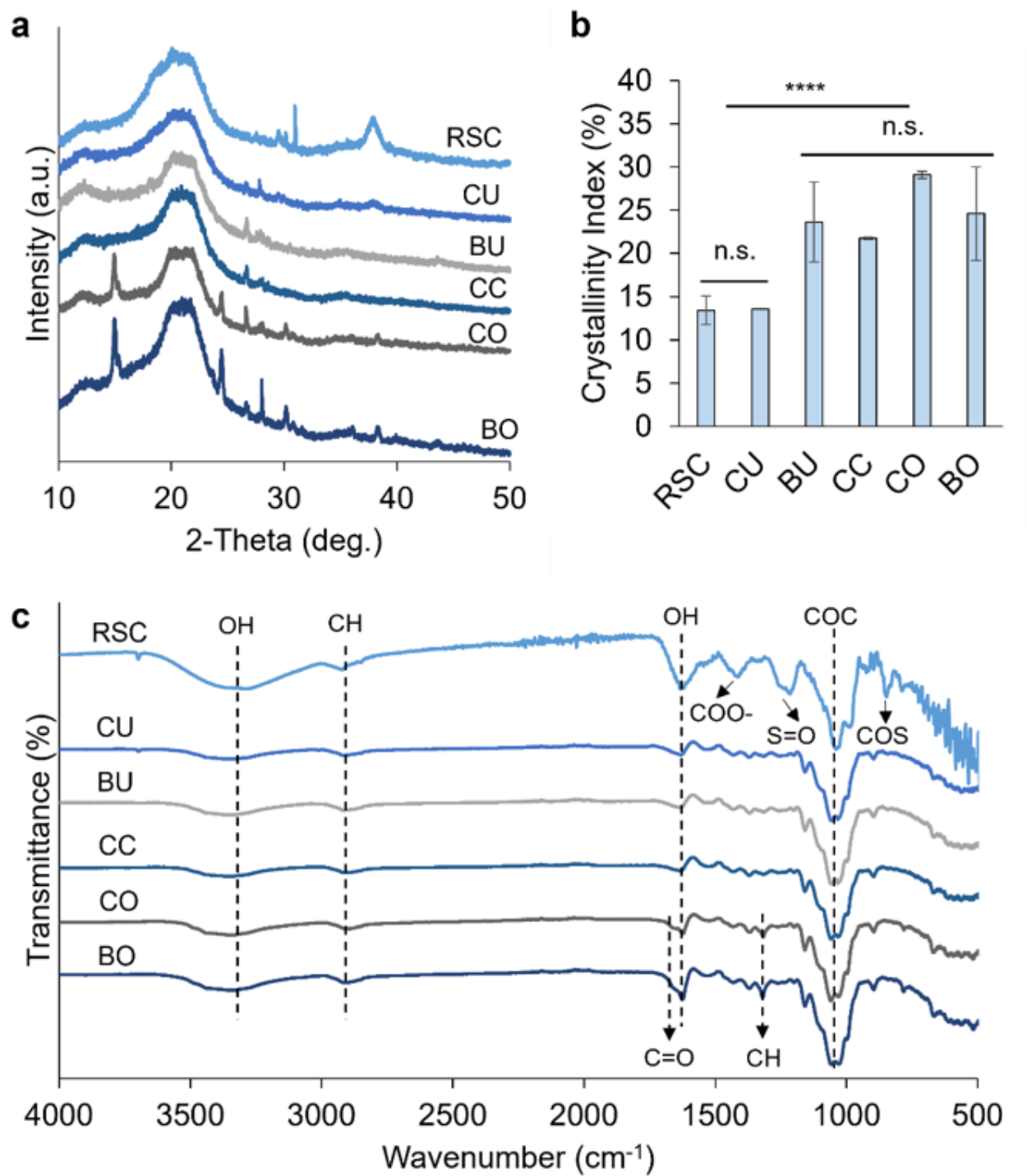


Figure 4

Structural characteristics of raw seaweed cellulose (RSC) and seaweed cellulose microfibers (SCMF) produced by hydrated deep eutectic solvent (DES) treatment with 30% of choline chloride:urea (CU), betaine:urea (BU), choline chloride:citric acid (CC), choline chloride:oxalic acid (CO), and betaine:oxalic acid (BO). (a) X-ray diffraction (XRD) patterns, (b) crystallinity index (CrI), and (c) Fourier-transform infrared (FTIR) spectra. CrI data are shown as mean $\pm$ SD (n = 3). Statistical differences were analyzed using Tukey's test; **** p < 0.0001, n.s. not significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformationfinal.docx](#)