Extraction of Eutectogel-Carrageenan from *Kappaphycus alvarezii* using Natural Deep Eutectic Solvent (NADES) Assisted by Ultrasonication

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Abstract

The extraction process of carrageenan from red seaweed (*Kappaphycus alvarezii*) still predominantly employs conventional solvents, which leave residues that pose potential risks to human health and the environment. Natural Deep Eutectic Solvents (NADES) are green solvents that are biodegradable, biocompatible, and sustainable, offer a promising alternative. The process of extracting carrageenan polysaccharides using NADES enables simultaneous synthesis-extraction (SSE). This process operates through supramolecular interactions and facilitates in situ gel formation, resulting in a Marine Eutectogel based on carrageenan, referred to as Eutectogel-Carrageenan. This study aims to determine the optimal extraction conditions, in terms of temperature and time, for the characteristics of the Eutectogel-Carrageenan-NADES (ECN) produced, and to compare them with those of the Eutectogel-Carrageenan-Commercial (ECC). The research process includes sample preparation, NADES preparation, Eutectogel-Carrageenan extraction using NADES assisted by ultrasonication, and characterization. The optimal condition was established at 80°C for 60 minutes, yielding ECN with a moisture content of 18.93%, yield of 28.81% (db), and viscosity of 5.82 cP. Comparative analyses showed that ECN had a slightly lower sulfate content (3.96% db) and nearly identical functional group spectra compared to ECC, along with rheological properties of lower rigidity and a lower melting point. These findings confirm that ECN produced under optimal NADES-assisted ultrasonication exhibits comparable quality to ECC. This study demonstrates, for the first time, feasibility of simultaneous synthesis–extraction of carrageenan eutectogels using NADES, offering a sustainable approach with concrete prospects for food, pharmaceutical, and biomedical applications.

Keywords: green solvents, in situ gel formation, polysaccharides, red seaweed, simultaneous synthesis-extraction

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Introduction

Indonesia's downstream seaweed industry is strengthened by increasing its added value into semi-finished products like carrageenan. The Government of the Republic of Indonesia has also pursued this through Presidential Regulation No. 33 of 2019 on the National Seaweed Industry Development Roadmap. The added value of carrageenan needs to be enhanced by investing in its processing techniques, aiming for optimal utilization by both the organic food and non-food industries. In the food industry, carrageenan is used as a stabilizer in ice cream, a gelling agent in jelly, a thickener in milk and sauces, and an emulsifier for water and oil (Devi *et al.*, 2020). In the non-food industry, carrageenan is an emulsifier in cosmetics, pharmaceuticals, paint, and textiles, as well as a stabilizer in ceramics, animal feed, and more (Shen & Kuo, 2017).

Between 2012 and 2016, carrageenan was reviewed and removed from the list of allowed organic food additives due to concerns over its potential carcinogenicity and inflammatory effects (FSM, 2016). Carrageenan is still considered a synthetic substance because of its extraction process, which is reviewed every five years (Dixon, 2016). Most carrageenan extraction processes still rely on conventional methods, which depend on solvent characteristics, thermal treatments, and mechanical agitation/mixing. Conventional solvents such as HCl, KOH, and $Ca(OH)_2$ have several drawbacks, including toxic residues harmful to the environment, the risk of thermal degradation, long processing times, and higher

production costs (Hernanndez-Carmona *et al*., 2013). These methods can also affect the qualitative characteristics or biological activity, as well as the quantitative yield of the compounds produced. Therefore, green extraction solvents and technologies are necessary, with a focus on the targeted compounds (Ummat *et al*., 2021). Green extraction uses alternative, natural solvents such as green solvents that reduce energy consumption and ensure the produced extract is safe and of high quality (Chemat *et al*., 2012).

Deep Eutectic Solvents (DES) are environmentally friendly, versatile alternative solvents composed of mixtures of compounds that when combined, exhibit a lower melting point than the individual components (Mcreynolds *et al*., 2022; Pari *et al*., 2025; Ramadhan *et al*., 2024). When the elements of a DES consist of primary metabolites such as choline derivatives, amino acids, organic acids, sugars, and polyols, it is referred to as Natural DES (NADES). NADES in extraction processes is more sustainable, relatively low-cost, safe, and liquid at room temperature, and its viscosity can be easily adjusted (Paiva *et al*., 2014). NADES serves as a functional liquid medium for dissolving both natural and synthetic chemicals, being biodegradable, biocompatible, exhibiting diverse polarities, and recyclable, while consistently providing reliable extraction results (Liu *et al*., 2018).

The mechanical extraction process utilizing ultrasonication, which is widely considered a green extraction technique due to its reduced energy demand, lower processing temperature, and shorter extraction time, offers several advantages (Chemat *et al*., 2017; Ummat *et al*., 2021). Ultrasonication has been widely applied for polysaccharide extraction, such as native agar (Uju *et al*., 2018), ulvan (Ramadhan *et al*., 2022), alginate and carrageenan (Youssouf *et al*., 2017), as well as for sonication pretreatment (Din *et al*., 2019). Ultrasonication is generally employed as a pretreatment method in protein extraction, as it can disrupt cell matrices to enhance extraction efficiency (Rahman & Lamsal, 2021). Therefore, ultrasonication can serve as an effective pretreatment to disrupt seaweed cell matrices, thereby improving the extraction yield.

The NADES system is integrated into a polymeric gel design known as eutectogel. Eutectogel represents a unique type of gel derived from eutectic mixtures, formed through cross-linking reactions between NADES/DES and other materials (Nicolau *et al*., 2024). Eutectogels are applied as smart materials in various fields, including drug delivery systems in biomedicine, organic electronics, supercapacitors, wearable sensors, CO_2 capture, and energy technologies, as well as in synthesis and catalysis. Research on eutectogels is still in its early stages, necessitating further exploration of new properties and applications. Eutectogels are classified into three types: eutectogel-s, eutectogel-p, and supramolecular eutectogels. The physical properties of supramolecular eutectogels are distinct, characterized by non-covalent interactions such as hydrogen bonding, electrostatic interactions, and others (Wang *et al*., 2021).

Supramolecular eutectogels are formed by combining DES/NADES with gelators synthesized at room or elevated temperatures. The comprehensive formation concept of supramolecular eutectogels is primarily influenced by the structural properties of the gelator (Marullo *et al*., 2018). For example, the gelator 1,3:2,4-dibenzylidene-D-sorbitol (DBS), when used at concentrations between 4% and 10%, can form an effective network throughout the matrix, significantly enhancing thermal stability up to 111°C (Ruiz-Olles *et al*., 2019). Previous studies have predominantly focused on low-molecular-weight gelators such as amino acids, sugars, nucleosides, amide derivatives, and organic salts (Souza *et al*., 2024). However, the use of bio-based gelators remains underexplored.

Polysaccharide-based eutectogels such as those derived from xanthan gum, have been investigated by Xia *et al*. (2020) and Zeng *et al*. (2020) using NADES systems like ChCl:Glycerol and ChCl:Xylitol. Polysaccharides sourced from aquatic resources such as carrageenan from *Kappaphycus alvarezii*, hold potential as gelators. Das *et al*. (2016) explored carrageenan extraction using DES including ChCl:Glycerol, ChCl:Ethylene Glycol, and ChCl:Urea, but their study focused on producing purified carrageenan rather than eutectogels. Carrageenan extraction was conducted at 85°C for 1 hour in that study. However, optimizing extraction temperature and duration using NADES has not been thoroughly addressed. To the best of our knowledge, no report has demonstrated the simultaneous synthesis-extraction of carrageenan eutectogels from *K. alvarezii* using NADES, representing a critical gap that this study addresses. The objectives are to determine the optimal temperature and time conditions for extracting Eutectogel-Carrageenan-NADES (ECN) and to compare these characteristics with those of Eutectogel-Carrageenan-Commercial (ECC).

Materials and Methods

Materials and Equipment

The primary material used in this study was dried red seaweed (*K. alvarezii*) sourced from Sumenep, Indonesia. Other materials included NADES components such as Choline Chloride and Glycerol (Himedia, Mumbai, India), 99% Isopropanol (Cliger,

Jakarta, Indonesia), and pure commercial carrageenan (IndoGum, Jakarta, Indonesia). The extraction process utilized equipment such as a Pioneer analytical balance (OHAUS, Parsippany, United States), an ultrasound cleaner bath (Skymen, Shenzhen, China), and a magnetic stirrer MS-H280-Pro (DLAB, Beijing, China). Analytical instruments included a DVPlus viscometer (AMETEK Brookfield, Middleboro, United States), FTIR-ATR Nicolet iS50 (ThermoFisher Scientific, Waltham, United States), DHR1 rheometer (TA Instrument, New Castle, United States), and DSC-60A Differential Scanning Calorimetry DSC-60A (Shimadzu, Kyoto, Japan).

Methods

Preparation of *K. alvarezii*

The sample used in this study was dried *K. alvarezii* seaweed. The sensory characteristics of the prepared samples were evaluated according to the Indonesian National Standard (SNI 2690:2023) on Dried Seaweed - Quality Standards and Processing. The sample was washed three times with water to remove impurities and, foreign materials (e.g., net residues, ropes, sand), then sorted to obtain fresh, clean seaweed with a bright, species-specific thallus color. Following four days of drying at room temperature, the samples were re-sorted to select seaweed exhibiting uniform dryness, firm and resilient texture, and resistance to breakage. The sample was ground using a disk mill to obtain finely powdered dried seaweed.

Preparation of NADES

The NADES preparation in this study comprises a mixture of choline chloride and glycerol, with a molar ratio of 1:2 (w/v), as referenced in Abbott *et al*. (2005). The reaction involves the interaction between the components, where choline chloride serves as the hydrogen bond acceptor, while glycerol acts as the hydrogen bond donor, resulting in the formation of a eutectic mixture solution. The mixture was heated to 80 °C under continuous stirring with a magnetic stirrer until a clear and homogeneous NADES solution was obtained.

Extraction of Eutectogel-Carrageenan using NADES assisted by Ultrasonication

The carrageenan extraction procedure was adapted from Das *et al*. (2016) with modifications. In contrast to their single-condition extraction at 85°C for 60 min, we applied a factorial design with three temperatures (80, 90, and 100°C) and three extraction times (60, 90, and 120 min). In addition, a 5-minute ultrasonication pretreatment at room temperature was introduced to enhance cell disruption and solvent penetration. Unlike Das *et al*. (2016), who focused on purified carrageenan, our protocol enabled simultaneous synthesis-extraction (SSE), directly yielding eutectogel-carrageenan.

The dried fine powder of *K. alvarezii* samples was extracted using a NADES solution at a ratio of 1:20 (w/v) in a beaker glass. The samples were dissolved in ChCl:Glycerol and subjected to pretreatment via ultrasonication at room temperature for 5 minutes. The extraction process for Eutectogel-Carrageenan employed NADES under two variable parameters: extraction temperature and time. The extractions were conducted at temperatures of 80, 90, and 100°C, with durations of 60, 90, and 120 minutes. This process yielded carrageenan polysaccharides that interacted simultaneously with NADES components through supramolecular mechanisms, facilitating the formation of a gel network.

These supramolecular interactions enabled a simultaneous synthesis-extraction approach, which resulted in the production of carrageenan-based gel materials. Simultaneous synthesis-extraction (SSE) is a multifunctional method that promotes combination and efficiency within a straightforward process. Subsequent steps included primary filtration using a 300-mesh nylon filter, followed by precipitation with 99% isopropanol at a 1:2 (v/v) ratio, and storage in a refrigerator for 24 hours. The precipitated samples underwent a secondary filtration step, during which in situ gel formation occurred, directly yielding the gel material referred to as Eutectogel-Carrageenan-NADES (ECN). The final step involved drying the gel in an oven at 50°C for 12 hours to enhance the stability of the Eutectogel properties.

Analysis Procedures

Moisture Content

The moisture content was determined by weighing a 1 g sample of ECN and placing it into a pre-weighed porcelain dish. The dish containing the sample was then heated in an oven at 105°C for 6 hours. After heating, the dish was placed in a desiccator to cool and was subsequently weighed using an analytical balance until a constant weight was achieved (AOAC 2005). The moisture content was calculated using the following formula:

$$\text{Moisture Content (\%)} = \frac{(B - C)}{(B - A)} \times 100\%$$

Description:

A = weight of the dish (g)

B = weight of the dish + initial sample (g)

C = weight of the dish + final sample (g)

Yield

The yield of the extracted carrageenan was calculated based on the ratio of the weight of the obtained extract to the weight of the dried seaweed (Tsubaki *et al*., 2016). The yield value was determined on a dry basis (%db). The carrageenan yield from ECN was calculated using the following formula:

$$\text{Yield (\%wb)} = \frac{\text{Weight of ECN (g)}}{\text{Weight of Dried Seaweed (g)}} \times 100\%$$

$$\text{Yield (\%db)} = \text{Yield (\%wb)} - \text{Moisture Content of ECN (\%)}$$

Description:

%wb = wet basis

%db = dry basis

Viscosity

The viscosity analysis procedure was adapted from SNI 8391-1:2017 with modifications. Viscosity measurement began by dissolving 0.75% ECN in distilled water (w/v). Viscosity was measured using a DVPlus viscometer (AMETEK Brookfield). The reading was taken digitally, with the value displayed on the instrument's screen. The measurement was conducted using spindle number 1 at a rotational speed of 150 rpm.

Sulfate Content

The sulfate content analysis procedure was also adapted from SNI 8391-1:2017 with modifications. The sulfate content was determined by adding 1 g of the Eutectogel-Carrageenan sample into an Erlenmeyer flask, followed by the addition of 10 mL of 0.1 N HCl. The mixture was heated at boiling temperature for 15 minutes. Then, 10 mL of 0.25 M BaCl2 solution was added and the mixture was heated in a water bath for 5 minutes. After cooling for 5 hours, the resulting precipitate was filtered through ash-free filter paper and washed with boiling distilled water until chloride ions were removed. The precipitate was then burned in a furnace at 700°C for 1 hour. The weight of white ash represents the weight of barium sulfate (BaSO4). The sulfate content on a dry basis (%db) of Eutectogel-Carrageenan was calculated using the following formula:

$$\text{Sulfate Content (\%wb)} = \frac{(C - B)}{A} \times 0{,}4116 \times 100\%$$

$$\text{Sulfate Content (\%db)} = \frac{\text{Sulfate Content (\%wb)}}{100 - \text{Moisture Content (\%)}} \times 100\%$$

Description:

A = weight of the sample (g)

B = weight of the empty/initial dish (g)

C = weight of the final dish (g)

Functional Groups

Functional group analysis was performed using Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance (FTIR-ATR). The procedure for functional group analysis followed the method of Das *et al*. (2016) with modifications. The functional group analysis of the Eutectogel-Carrageenan gel sample was conducted by obtaining the infrared spectrum and was recorded within the wave number range of 4000 cm^{-1} to 400 cm^{-1}.

Rheology

The rheological analysis procedure follows Das *et al*. (2016) method with modifications. The rheometer DHR1 (TA Instrument, New Castle, DE, USA) with the oscillation frequency method was used to analyze the rheology of Eutectogel-Carrageenan. Two types of oscillation frequency tests were performed: the storage modulus (G') and the loss modulus (G") at a temperature of 25°C. The testing parameters included a frequency range of 0.1 to 10 Hz and a strain of 0.1%.

Thermal Profile

A Differential Scanning Calorimetry DSC-60A instrument (Shimadzu, Kyoto, Japan) was employed for thermal profiling analysis. The instrument was equipped with a temperature range of RT-500 °C and a heating rate capability of 0.01-100°C/min. It had a temperature resolution of 0.001 °C and a heat-flow resolution of 0.1 µW. Baseline reproducibility was ±5 µW, with temperature reproducibility of ±0.1 °C and enthalpy reproducibility within ±1%. The system was operated under nitrogen purge gas, with a maximum operating pressure of 0.2 MPa. The thermal profiling procedure using DSC follows the method of Sedayu *et al*. (2020) with modifications. The Eutectogel-Carrageenan sample was weighed to 25 mg and heated in a nitrogen atmosphere. The temperature range applied was from 25°C to 500°C, with a heating rate of 10°C/min.

Data Analysis

The research data were statistically analyzed using Microsoft Excel, Statistical Product and Service

Solutions (SPSS) 25, and Origin Pro 2024b. All analyses were conducted in triplicate (n=3), and the generated data were statistically evaluated for normality and homogeneity before being assessed with variance analysis. The analysis of moisture content, yield, and viscosity of ECN was conducted using a Factorial Completely Randomized Design. The two factors tested were different temperature and time conditions. The temperature factor consisted of three levels (80, 90, and 100°C), while the time factor had three levels (60, 90, and 120 min). The analysis of variance (ANOVA) was performed at a 95% confidence level (α=0.05) to determine significant differences. If significant differences were observed (significance value p<0.05), a Tukey HSD test was conducted for further analysis. A T-test was applied to compare the sulfate content of the selected ECN treatment and ECC. This comparison was analyzed using an independent T-test. Decision-making was based on the significance value at a 95% confidence level or a 5% significance level (α=0.05).

Results and Discussion

Strategy of This Current Research

Eutectogels represent an emerging class of smart materials whose characteristics are still being refined through advanced formulation and methodological integration, as well as their potential applications. Current research prioritizes the development of eutectogels using natural gelators combined with efficient and environmentally friendly methods. In line with this focus, we aim to explore and establish a simple, eco-friendly approach for eutectogel synthesis. An illustrative schematic of this current research framework is presented in Figure 1.

Eutectogels can be prepared through several approaches (Ding *et al*., 2025; Hu *et al*., 2025). First, polymeric eutectogels are obtained by introducing polymeric gelators (e.g., polyvinyl alcohol, polyacrylamide) into NADES/DES systems, forming chemically or physically cross-linked networks. Second, supramolecular eutectogels rely on the self-assembly of low-molecular-weight gelators such as amino acids, sugars, or organogels within eutectic solvents, stabilized by hydrogen bonding and π–π interactions. Third, polysaccharide-based eutectogels employ naturally derived biopolymers such as xanthan gum, alginate, or carrageenan that interact with NADES components through non-covalent forces. In this study, our framework (Figure 1) emphasizes the polysaccharide-based route, integrating green extraction via NADES with ultrasonication pretreatment, enabling simultaneous synthesis-extraction (SSE) of carrageenan to directly form marine eutectogels. This approach simplifies processing, reduces solvent use, and offers a sustainable strategy compared to conventional multi-step gelation.

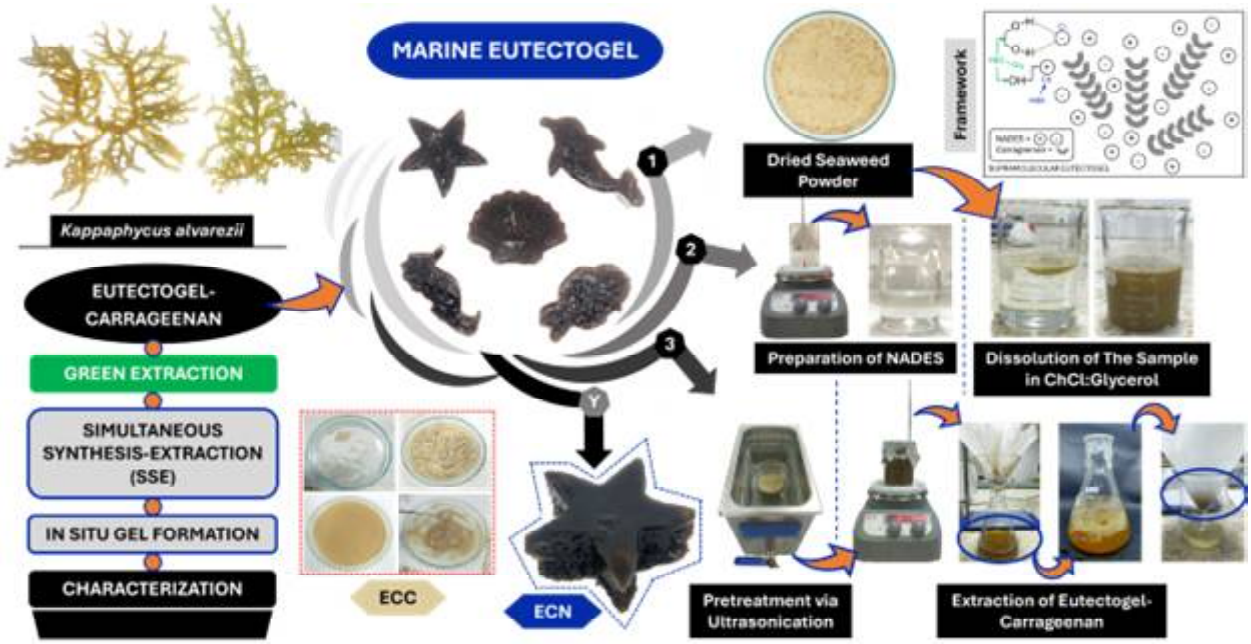

Figure 1. Schematic of this current research.

Characteristics of ECN

Moisture Content

The difference in weight of ECN before and after drying in the gravimetric process was used to calculate the moisture content. The results of the moisture content analysis of ECN are presented in Figure 2. Analysis of variance indicated that the interaction between temperature and time had a significant effect ($p<0.05$) on the moisture content of the ECN produced. The temperature factor also had a significant effect ($p<0.05$), while the time factor did not have a significant effect ($p>0.05$). The treatment at 80°C and 90°C showed no significant difference; however, a significant difference was observed when compared to the treatment at 100°C. The lowest moisture content (15.31%) was recorded under the treatment at 80°C for 120 minutes, whereas the highest moisture content (19.87%) was obtained under the treatment at 100°C for the same duration.

The moisture content exceeded the carrageenan quality specification of 12% (FAO, 2014). The gel system of carrageenan or ECN presents a distinct condition. Functional groups of proteins and polysaccharides, as well as the porous structure of the gel network, can bind water. As carrageenan content increases, more water is bound, affecting the gel network (Shen & Kuo, 2017). Three types of water are present in the gel: free water, frozen bound water, and non-frozen bound water. Free water evaporates easily, frozen bound water freezes below 0°C, and non-frozen bound water is strongly attached, resisting freezing (Xia *et al*., 2020).

The lower moisture content observed could be due to drying conditions, including temperature and time, during the ECN drying process and storage-related syneresis. Carrageenan's double-helix structure holds water tightly at lower temperatures. Low temperatures in freezing conditions negatively impact the gel's microstructure, potentially reducing its water-holding capacity, although it remains favorable overall. Carrageenan gel syneresis ranged from 7.80% to 21.50% after 10 days of storage (Chan *et al*., 2013). Increased syneresis may also be caused by lower drying temperatures. Research by Faria *et al*. (2014) demonstrated that carrageenan gel syneresis at drying temperatures between 90°C and 40°C ranged from approximately 10.30% to 15.70%. Additionally, the hygroscopic nature of the NADES component (ChCl) may introduce water from contaminants or biomass within the gel system (Weerasinghe *et al*., 2024). This higher moisture retention, beyond the FAO specification, reflects the hygroscopic contribution of ChCl in NADES, which introduces additional bound water domains in the carrageenan network.

The hygroscopic choline chloride in NADES likely competes with carrageenan hydroxyl and sulfate groups for water binding, shifting the balance between free and bound water domains. This competitive hydration reduces water activity within the gel matrix, which explains the distinct moisture retention profile compared with conventional carrageenan gels. Overall, ECN moisture retention reflects the balance between carrageenan helix packing and the hygroscopic nature of NADES, which together determine gel stability against syneresis.

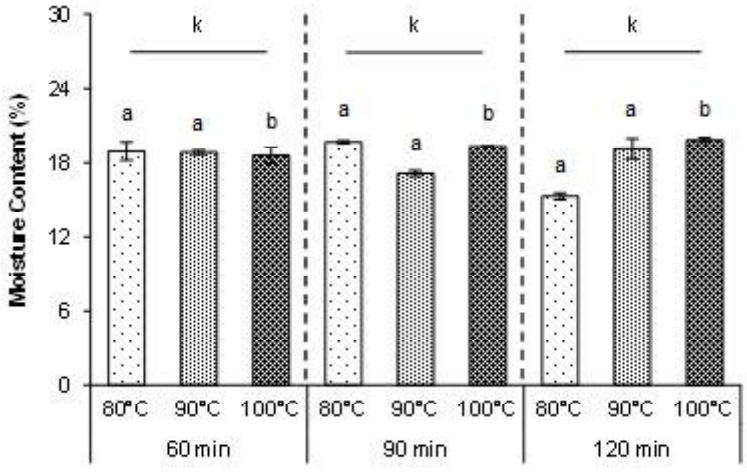

Figure 2. The moisture content of ECN under different extraction conditions. Different superscript letters for temperature (a, b, c) and time (k, l, m) indicate statistically significant differences ($p<0.05$).

Yield

Yield is an important parameter for assessing the effectiveness and efficiency of an extraction process. The yield calculation in this study was based on the results after moisture content analysis. The yield analysis results for ECN (%db) are presented in Figure 3. The dry basis yield analysis (%db) aimed to determine the carrageenan content in the ECN without considering the moisture content. Analysis of variance indicated that both the temperature and time factors significantly affected the yield of ECN (p<0.05). However, the interaction between temperature and time did not have a significant effect (p>0.05). Tukey's post-hoc test revealed that the treatments at temperatures of 80, 90, and 100°C, as well as extraction times of 60, 90, and 120 minutes, each had a significant effect on the yield of ECN obtained. Yields ranged from 28.81% to 57.26%, with the lowest at 80°C for 60 minutes (28.81%) and the highest at 100°C for 120 minutes (57.26%). As both temperature and extraction time increased, the yield of ECN also increased, as shown in Figure 3. Despite the high yield, residual NADES interacted with carrageenan to form ECN, though NADES (ChCl:Glycerol) is non-toxic, indicating no adverse effects of its application (Das *et al*., 2016).

Generally, the higher yield, compared to previous reports on carrageenan extraction, is also attributed to pre-treatment. Specifically, ultrasonication at room temperature for 5 minutes facilitates the breakdown of *K. alvarezii* seaweed cell walls, enhancing extraction efficiency. The solvent then interacts with polysaccharides, and ultrasonic waves help by enhancing solvent penetration, improving extraction efficiency (Uju *et al*., 2018). Compared to Das *et al*. (2016), the yields in this study were slightly lower. Their study yielded 30.93% using pure ChCl:Glycerol at 85°C for 60 minutes and 60.25% with 10% hydrated ChCl:Glycerol at 85°C for 60 minutes. Yield increase was attributed to ultrasound pretreatment, which disrupted seaweed cell walls and enhanced solvent penetration, consistent with previous reports on polysaccharide extraction. The efficiency also reflects the polarity and hydrogen-bonding capacity of ChCl:Gly NADES, which facilitates carrageenan solubilization. Unlike conventional DES-based extraction that isolates purified carrageenan, our method simultaneously produces eutectogel, representing a distinct advantage in process integration. Therefore, the yield of carrageenan is influenced by the type of solvent and extraction conditions, which leads to varying results. The ChCl:Glycerol solvent as a NADES, also has hydrophilic and high polarity properties (Liu *et al*., 2018), which gives it excellent hydrogen-donating and accepting capabilities (Sakti *et al*., 2019) within the carrageenan natural product matrix. However, yield alone does not necessarily reflect functional gel quality, as part of the mass increment may arise from co-precipitated NADES residues.

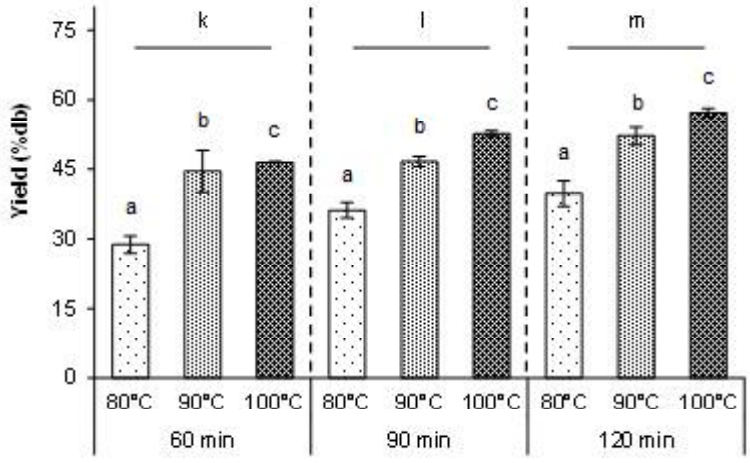

Figure 3. The yield of ECN under different extraction conditions. Different superscript letters for temperature (a, b, c) and time (k, l, m) indicate statistically significant differences (p<0.05).

Viscosity

The quality and functionality of carrageenan as a primary component in gel formation, thickening agents, and stabilizers require viscosity analysis. The results of the ECN viscosity analysis are depicted in Figure 4. Analysis of variance indicated that the all factors including temperature and extraction time, and their interaction significantly affected the viscosity values of ECN (p<0.05). The treatments at 80, 90, and 100°C exhibited significant differences among each other. In contrast, the treatments at 60 minutes and 90 minutes

showed no significant difference, whereas both were significantly different from the treatment at 120 minutes. The viscosity analysis revealed a trend where higher extraction temperatures and longer durations led to a decrease in ECN viscosity. The lowest viscosity (4.66 cP) was observed at 90°C for 120 minutes, while the highest viscosity (5.82 cP) was achieved at 80°C for 60 minutes.

The hydrophilic nature of carrageenan allows water molecules to bind around the sulfate groups, and as the temperature increases, viscosity decreases due to water evaporation (Heriyanto *et al*., 2018). Additionally, a hydrated DES system with 35% water (water in DES) can be employed to maintain the structural integrity of the DES mixture during the extraction process (Ferreira *et al*., 2021). Importanly, the extraction condition of 80°C for 60 minutes was selected as the optimal treatment, considering not only the viscosity values but also compliance with the minimum viscosity specification for carrageenan, set at 5 cP (FAO, 2014). The decline in viscosity at harsher conditions is consistent with chain scission and sulfate depletion, reducing network entanglement. The observed viscosity reduction at higher temperatures and longer times is consistent with NADES-sulfate interactions that disrupt carrageenan helix aggregation, leading to fewer entanglements and a softer gel matrix.

At the molecular level, NADES components screen electrostatic repulsion between sulfate groups and disrupt inter-helix hydrogen bonding, resulting in partial helix loosening and reduced network entanglement. This explains the lower viscosity under harsher conditions and the softer viscoelasticity of ECN compared with ECC.

Comparison of the Selected ECN Characteristics with ECC

The extraction condition of 80°C for 60 minutes was selected as optimal as it maximized viscosity, reflecting the gel's functional integrity, while also minimizing energy requirements. ECN produced under these conditions was compared to ECC, a gel made from pure commercial carrageenan and ChCl:Glycerol solvent, adjusted to match the moisture content of ECN (18.93%). Comparative analyses included sulfate content, functional groups, rheological properties, and thermal profiles.

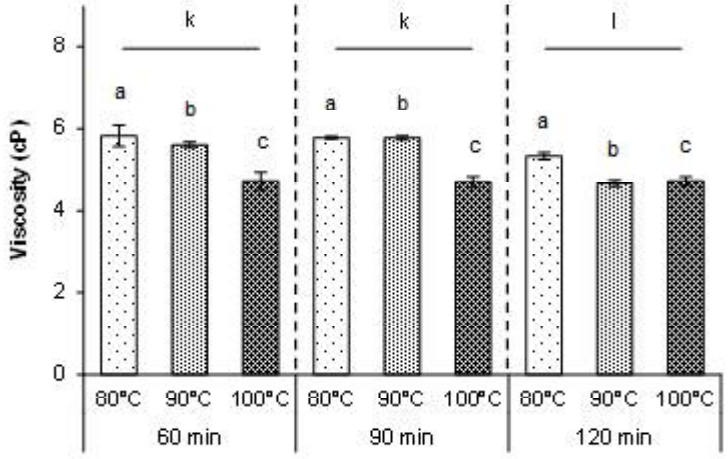

Figure 4. The viscosity of ECN under different extraction conditions. Different superscript letters for temperature (a, b, c) and time (k, l, m) indicate statistically significant differences (p<0.05).

Sulfate Content

The analysis of sulfate content is essential to evaluate the physicochemical properties of ECN, particularly its correlation with viscosity. An independent T-test revealed no significant difference (p>0.05) in sulfate content between the selected ECN (3.96%) and ECC (4.37%) conditions. The observed sulfate content positively correlates with viscosity, whereby lower sulfate content corresponds to a lower viscosity value. The low sulfate content observed in both Eutectogel-Carrageenan samples may be influenced by the ChCl component in ChCl:Glycerol, a quaternary ammonium salt acting as an acceptor, and variations in extraction conditions. Strong interactions between sulfate groups and NADES can result in the partial loss of anions, thereby modifying the structure of the resulting polysaccharides (Das *et al*., 2016). The results of sulfate content analysis for the selected ECN and ECC samples are displayed in Figure 5.

The low sulfate content in carrageenan gels corresponds to an increased presence of 3,6-anhydro-D-galactose, reducing electrostatic repulsion and

potentially decreasing viscosity and gel strength due to structural modifications in the double-helix aggregation (Liu *et al.*, 2022). Other influencing factors include the type of carrageenan, the species and growth conditions of the seaweed, and the harvest age (Asikin & Kusumaningrum, 2019). Sulfate groups play a key role in gel formation and multifunctional applications, including drug delivery systems (Cunha & Grenha, 2016). Carrageenan sulfate groups are also suggested to assist in interactions like (meth)acrylic or other reactive functional organic compounds during eutectogel formation. The formation of eutectogel matrices involves cross-linking reactions and polymerization within eutectic mixtures (Nicolau *et al.*, 2024).

The observed lower sulfate content correlates with reduced viscosity and gel strength. This is consistent that sulfate depletion modifies helix aggregation. In our system, NADES likely mediates ion-exchange or shielding effects, selectively interacting with sulfate groups and thus modulating gel packing-an effect not evident in conventional solvent extractions.

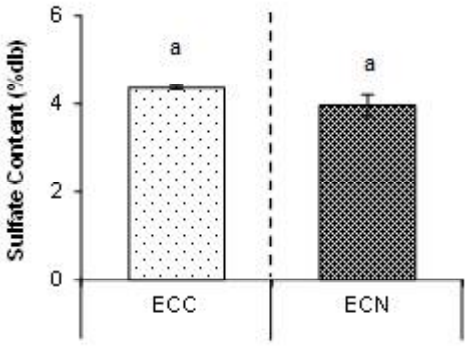

Figure 5. The sulfate content of Eutectogel-Carrageenan-Commercial (ECC) and Eutectogel-Carrageenan-NADES (ECN). Identical superscript letters (a, b) for both treatments indicate significant difference (p<0.05).

Functional Groups

The analysis of functional groups was conducted on the selected ECC and ECN samples to identify specific functional groups within their structures. The analysis of functional groups, as shown in Figure 6, reveals that both samples exhibit almost identical spectral patterns. The functional groups detected in the ECC sample (a) include O-H groups at an absorption band of 3320 cm^{-1}, C-H groups (2929 cm^{-1}), COO^- groups (1637 cm^{-1} to 1419 cm^{-1}), S=O groups (1235 cm^{-1}), C-O groups (1024 cm^{-1} to 1020 cm^{-1}), and C-O-S groups (845 cm^{-1}). Similarly, the functional groups identified in the selected ECN sample (b) comprise O-H groups at 3304 cm^{-1}, C-H groups (2937 cm^{-1}), COO^- groups (1651 cm^{-1} to 1476 cm^{-1}), S=O groups (1206 cm^{-1}), C-O groups (1040 cm^{-1} to 1030 cm^{-1}), and C-O-S groups (863 cm^{-1}). Additionally, several new absorption bands were observed at distinct wavenumbers: C-H (2881 cm^{-1}) in sample (b), COO^- at 1370 cm^{-1} (a) and 1416 cm^{-1} (b), C=S at 1150 cm^{-1} (a), and C-O at 924 cm^{-1} (a) as well as 1109 cm^{-1}, 994 cm^{-1}, 954 cm^{-1}, and 923 cm^{-1} (b).

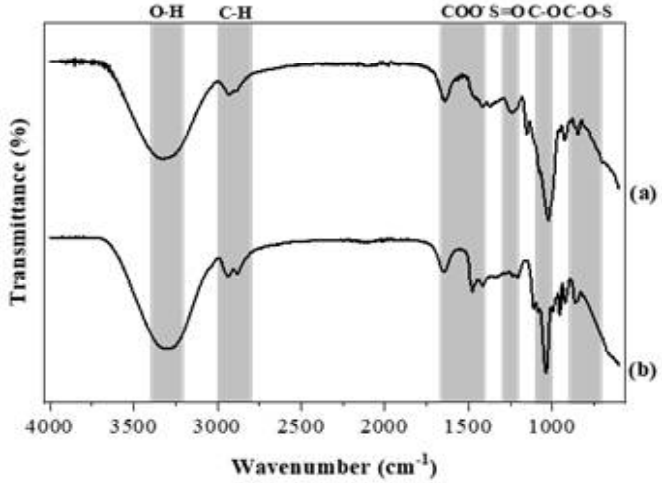

Figure 6. The Fourier transform infrared spectroscopy spectra of ECC (a) and selected ECN (b).

The functional groups of carrageenan exhibit several absorption bands, including O-H groups (3400 cm^{-1} to 3200 cm^{-1}), C-H groups (2935 cm^{-1} to 2828 cm^{-1}), COO^- groups (1640 cm^{-1} to 1418 cm^{-1}), S=O groups (1260 cm^{-1} to 1220 cm^{-1}), C-O groups (1068 cm^{-1} to 1010 cm^{-1}), and C-O-S groups (900 cm^{-1} to 700 cm^{-1}) (Bakar *et al*., 2020; Jumaah *et al*., 2015; Martín-del-Campo *et al*., 2021; Sun *et al*., 2014). Absorption bands at 1069 cm^{-1} to 1068 cm^{-1} and 927 cm^{-1} to 915 cm^{-1} (C-O groups) indicate the presence of 3,6-anhydro-D-galactose. Furthermore, absorption bands around 848 cm-1 to 835 cm^{-1} (C-O-S groups) signify the presence of D-galactose-4-sulfate, a key marker of polysaccharides characteristic of the kappa-carrageenan structure (Arzani *et al*., 2020; Das *et al*., 2016; Ferdiansyah *et al*., 2023).

The selected ECN sample (b) exhibited stronger O-H group absorption intensity compared to the ECC sample (a), indicating enhanced hydrogen bonding interactions with electronegative atoms. According to Zeng *et al*. (2020), polysaccharides such as xanthan gum, when integrated into NADES for eutectogel formation, modify the supramolecular hydrogen bonding network, altering absorption intensities. The absorption band of C-O or C-O-C groups around 1023 cm^{-1} indicates interactions between negatively charged structures and the O-H groups (Prasetyaningrum *et al*., 2024). The positively charged choline (Ch^+) is intercalated among polysaccharide chains, impacting the intensity of hydrogen bonding (Marullo *et al*., 2023). Additionally, the absorption band detected around 1040 cm^{-1} to 1030 cm^{-1} (b) falls within the range of glycosidic bond absorption (Sedayu *et al*., 2020).

Functional group analysis (Figure 6) revealed shifts and increases in absorption bands. The S=O group (1206 cm^{-1}) (b) and C-O-S group (863 cm^{-1}) (b) suggest structural modifications in carrageenan due to NADES interactions. This condition is closely associated with the high infrared absorption intensity of the C-O and C-O-C groups, suggesting an increased presence of 3,6-anhydro-D-galactose. The increase in 3,6-anhydro-D-galactose correlates with enhanced gel strength and reduced sulfate content in carrageenan (Anisuzzaman *et al*., 2013). The absorption band around 1206 cm^{-1} (b) can be attributed to the symmetric S=O group, related to the deformation of the C-O-C group in the pyranose ring (Popescu *et al*., 2019). The absorption band range around 1637 cm^{-1} (a) and 1651 cm^{-1} (b) indicates the asymmetric COO- group and the deformation of water (H_2O), which is associated with the vibration of the O-H group. The absorption bands around 1419 cm^{-1} (a) and 1476 cm^{-1} (b) are attributed to the symmetric COO- group from the carboxylate ($-COO^-$) salt (Jumaah *et al*., 2015). These FTIR spectral shifts, particularly the stronger O-H absorption and S=O band modifications due to NADES-sulfate interactions, are consistent with the softer viscoelastic profile of ECN observed in rheology, confirming that the supramolecular interactions modulate carrageenan helix packing and gel rigidity.

Rheology

Rheology is the simulation and prediction of a material's mechanical response to flow and deformation conditions. Rheological analysis describes the reaction of a material to forces, pressure, and strain through several parameters, one of which is oscillation frequency. The results of the rheological analysis presented in Figure 7 demonstrate that both samples exhibit a storage modulus (G') significantly higher than the loss modulus (G"). The G' (Pa) values consistently dominate and increase at higher frequency ranges. These findings indicate that both samples exhibit strong gel behavior. The G' curve, positioned higher and diverging from the G" curve, suggests that both gels behave similarly to solids or strong gels (Horkay & Douglas, 2018). Consequently, both Eutectogel-Carrageenan (a) and (b) exhibit superior energy storage capacity compared to mechanical energy loss. The rheological analysis results for ECC and the selected ECN are presented in Figure 7.

The ECC sample (a) exhibits a G'/G" ratio of 12.47, which is higher than that of the selected ECN (b) with a G'/G" ratio of 3.95. A higher G'/G" ratio indicates that the gel sample possesses a higher rigidity, whereas a lower ratio suggests a softer characteristic (Flores *et al*., 2017). Therefore, in terms of flow properties, ECC (a) demonstrates a higher rigidity (solid-gel) compared to the selected ECN (b). The softer viscoelastic profile of ECN reflects weaker helix aggregation compared with ECC, consistent with the concept that solvent-mediated interactions can tune gel rigidity. This softness is advantageous for applications requiring spreadable or injectable gels, whereas ECC is more suited for rigid formulations. The high values of G' and G'' may be influenced by the high salt concentration in the carrageenan gel sample. The salt interactions can be stronger than the effects of the concentration or presence of carrageenan, resulting in a gel with increased stiffness and strength (Torres *et al*., 2016). The formation of polymer networks in carrageenan polysaccharides through physical aggregation (physical gel) results in a structure that is generally less ordered and weaker. However, there is regular aggregation of helical segments that form rigid chains. According to Nishinari, (2009) elevated values of G' and G" do not inherently signify a high gel strength in terms of deformation resistance.

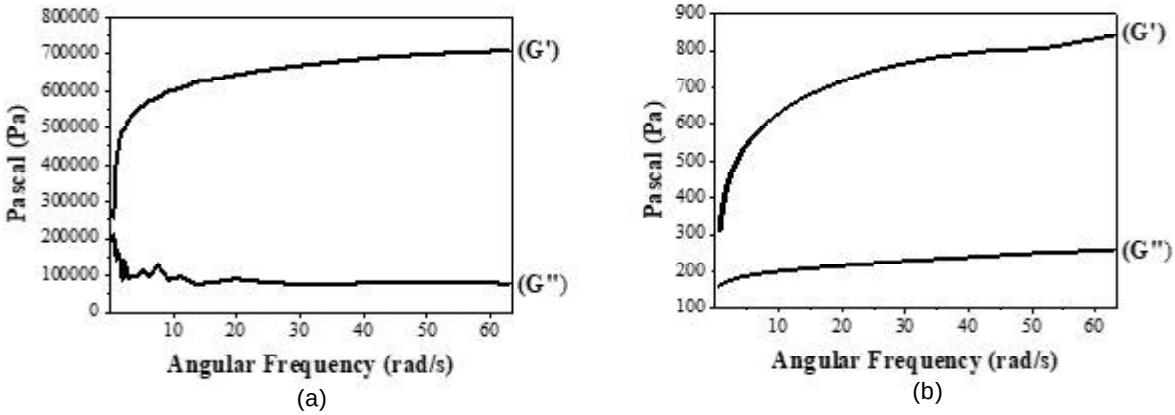

Figure 7. The rheology of ECC (a) and selected ECN (b). Description: G' (storage modulus) and G'' (loss modulus).

Carrageenan is considered a good natural gelator because its sulfated galactan chains form ordered double helices that aggregate into a three-dimensional network, entrapping water molecules. The main criteria of a good gelator include: (i) the ability to self-assemble via non-covalent interactions (e.g., hydrogen bonding, electrostatics), (ii) the formation of a stable 3D gel network where G' > G", (iii) sufficient rigidity or viscoelasticity depending on the application, and (iv) reproducible gelation under controlled conditions (Horkay & Douglas, 2018; Nishinari, 2009; Wang *et al.*, 2021). Based on these criteria, carrageenan demonstrated good gelator properties in both ECC and ECN, with ECC showing higher rigidity while ECN maintained softer viscoelastic behavior consistent with eutectogel formation. This softer viscoelastic profile is advantageous for spreadable or injectable formulations, whereas ECC is more suited for rigid gel applications. Besides sulfate-mediated electrostatic interactions, the proportion of 3,6-AG residues also governs the gel's viscoelasticity. Increased 3,6-AG favors ordered double-helix domains, which support stable network formation while maintaining the softer viscoelastic profile observed in ECN.

The softer viscoelastic behavior of ECN, reflected in its lower G'/G" ratio, is advantageous for applications that require deformable yet cohesive matrices, such as spreadable food gels, emulsified sauces, or injectable biomaterials. In contrast, the higher rigidity of ECC aligns with applications demanding structural stability, such as confectionery gels, dairy stabilizers, or packaging films. From a pharmaceutical perspective, softer eutectogels like ECN may facilitate drug loading and controlled release due to their flexible network, while rigid ECC-type gels are better suited for scaffolds or oral delivery matrices. Thus, rheological distinction between ECC and ECN highlights potential domain-specific advantages in both food and biomedical applications.

Thermal Profile

Thermal profile analysis examines the physical and chemical transitions of Eutectogel-Carrageenan biomaterials using differential scanning calorimetry. The DSC results presented in the thermogram (Figure 8) showed the presence of a melting point transition with increasing temperature. The ECC sample (a) displays two detected melting points at higher temperatures, specifically at 285.52°C and 428.67°C. The selected ECN sample (b) exhibits three melting points, at 122.13°C, 255.33°C, and 293.57°C. The three melting points of ECN (b) correspond to physical segment aggregation and hydrogen bonding during heating, resulting in multiple peaks. The 100°C melting point reflects dehydration and decomposition, influenced by COO^- group characteristics and water deformation (Figure 6), which enhance heat reactivity (Jamaludin *et al.*, 2017). The DSC thermogram is presented in Figure 8.

The endothermic melting points indicate water presence, which affects carrageenan gel stability and three-dimensional matrix formation (Mahmood *et al.*, 2014). Non-freezing bound water strongly interacts with eutectogel molecules, altering melting peaks (Xia *et al.*, 2020). Helical segment aggregation in carrageenan enhances gel network strength, further supported by Ch^+ ions acting as structural links, similar to K^+ions, thereby increasing gel strength and melting point (Iijima *et al.*, 2014; Nunez-Santiago & Tecante, 2007). The carrageenan concentration in eutectogels influences melting point, with lower concentrations reducing it (Zhao *et al.*, 2020). Variations in composition and synthesis methods between ECC (a) and ECN (b) result from differences in carrageenan content and interaction with NADES during extraction. Supramolecular interactions of NADES drive physical cross-linking, affecting gel properties and stability (Zeng *et al.*, 2020). The carrageenan as a stabilizer and gelling

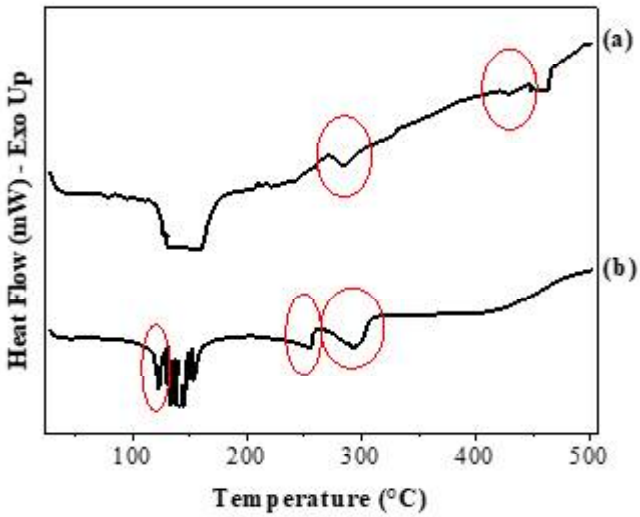

Figure 8. The DSC thermogram of the melting point (endothermic) of ECC (a) and selected ECN (b).

agent, is suggested to adapt well to the ChCl:Glycerol components, enabling the eutectogel to undergo thermal transitions at higher temperatures. This adaptation forms a more stable and organized supramolecular network, utilizing non-covalent bonds (Ruiz-Olles *et al*., 2019). The multiple endothermic peaks of ECN reflect stepwise disruption of bound-water and hydrogen-bond domains, consistent with its looser supramolecular network.

It should be noted that enthalpy values (ΔH) were not recorded in this study. Nevertheless, the presence of multiple low-temperature transitions in ECN indicates weaker cooperative hydrogen bonding and reduced network stability compared with ECC. This interpretation is consistent with its softer viscoelastic behavior. Future investigations incorporating enthalpy data will be valuable to strengthen the correlation between thermal transitions and supramolecular gel stability

Conclusion

The combination of extraction temperature and duration significantly influenced the physicochemical properties of the resulting Eutectogel-Carrageenan (ECN). At the optimal condition (80 °C for 60 min), ECN demonstrated comparable composition to ECC but with softer viscoelasticity and a lower melting point. These results confirm the mechanistic role of NADES in modulating carrageenan structure-function relationships: the choline-based NADES system interacts electrostatically with sulfate groups and through hydrogen bonding, thereby reducing electrostatic repulsion and altering helix packing. This supramolecular modulation leads to softer gel networks with distinct viscoelastic behavior compared to conventional carrageenan gels. The softer ECN is suitable for spreadable foods and biomedical gels, whereas the more rigid ECC is appropriate for solid-textured foods and packaging. Thus, the ECN platform widens the functional range of carrageenan-based gels. Importantly, this study provides the first demonstration of a one-pot, simultaneous synthesis-extraction (SSE) of polysaccharide-based eutectogels via a green NADES-ultrasonication system. This approach reduces process steps, eliminates toxic solvents, and offers a sustainable pathway for the seaweed industry. The extraction condition (80 °C/60 min) is also compatible with jacketed stirred tanks and centrifugal pumps, highlighting its feasibility for industrial scale-up. Future studies should optimize multi-response targets (yield-viscosity-stability), conduct storage and safety evaluations, and test continuous ultrasonication scale-up for food and biomedical translation.

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References

Abbott, A. P., Bell, T. J., Handa, S., & Stoddart, B. (2005). O-Acetylation of cellulose and monosaccharides using a zinc based ionic liquid. *Green Chemistry*, 7(10), 705-707. https://doi.org/10.1039/b511691k

Anisuzzaman, S. M., Bono, A., Samiran, S., & Farm, Y. Y. (2013). Influence of potassium hydroxide concentration on the

carrageenan functional group composition. *Developments in Sustainable Chemical and Bioprocess Technology*, 355-363. https://doi.org/10.1007/978-1-4614-6208-8_42

Arzani, L. D. P., Muhandri, T., & Yuliana, N. D. (2020). Karakteristik karagenan semi-murni dari rumput laut Kappaphycus striatum dan *Kappaphycus alvarezii*. *Jurnal Teknologi Dan Industri Pangan*, 31(2), 95-102. https://doi.org/10.6066/jtip.2020.31.2.95

Asikin, A. N., & Kusumaningrum, I. (2019). Karakteristik fisikokimia karaginan berdasarkan umur panen yang berbeda dari Perairan Bontang, Kalimantan Timur. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 22(1), 136-142. https://doi.org/10.17844/jphpi.v22i1.25890

Association of Official Analytical Chemist. (2005). Official Method of Official Analytical Chemist. Virginia: The Association of Official Analytical Chemist.

Badan Standardisasi Nasional. 2017. Karaginan murni (Refined Carrageenan) - Bagian 1: Kappa Karaginan - Syarat Mutu dan Pengolahan. SNI 8391-1:2017. Jakarta (ID): Badan Standardisasi Nasional.

Badan Standardisasi Nasional. 2023. Rumput laut kering - Syarat mutu dan pengolahan. SNI 2690:2023. Jakarta (ID): Badan Standardisasi Nasional.

Bakar, M. H. A., Azeman, N. H., Mobarak, N. N., Mokhtar, M. H. H., & Bakar, A. A. A. (2020). Effect of active site modification towards performance enhancement in biopolymer k-carrageenan derivatives. *Polymers*, 12(9), 1-13. https://doi.org/10.3390/POLYM12092040

Chan, S. W., Mirhosseini, H., Taip, F. S., Ling, T. C., & Tan, C. P. (2013). Comparative study on the physicochemical properties of k-carrageenan extracted from *Kappaphycus alvarezii* (doty) doty ex Silva in Tawau, Sabah, Malaysia and commercial k-carrageenans. *Food Hydrocolloids*, 30(2013), 581-588. https://doi.org/10.1016/j.foodhyd.2012.07.010

Chemat, F., Rombaut, N., Sicaire, A., Meullemiestre, A., & Abert-vian, M. (2017). Ultrasound assisted extraction of food and natural products, mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, 34(2017), 540-560. https://doi.org/10.1016/j.ultsonch.2016.06.035

Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: concept and principles. *International Journal of Molecular Sciences*, 13(7), 8615-8627. https://doi.org/10.3390/ijms13078615

Cunha, L., & Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs*, 14(3), 42. https://doi.org/10.3390/md14030042

Das, A. K., Sharma, M., Mondal, D., & Prasad, K. (2016). Deep eutectic solvents as efficient solvent system for the extraction of k-carrageenan from *Kappaphycus alvarez*ii. *Carbohydrate Polymers*, 136(2016), 930-935. https://doi.org/10.1016/j.carbpol.2015.09.114

Devi, F. P., Riyadi, D. N., Kurniawansyah, F., & Roesyadi, A. (2020). Produksi kappa karaginan dari rumput laut (*Eucheuma cottonii*) dengan metode semi-refined carrageenan. *Journal of Fundamentals and Applications of Chemical Engineering*, 1(1), 1-4. https://doi.org/10.12962/j2964710X.v1i1.12724

Din, S. S., Chew, K. W., Chang, Y. K., Show, P. L., Phang, S. M., & Juan, J. C. (2019). Extraction of agar from *Eucheuma cottonii* and *Gelidium amansii* seaweeds with sonication pretreatment using autoclaving method. *Journal of Oceanology and Limnology*, 37(3), 871-880. https://doi.org/10.1007/s00343-019-8145-6

Ding, K., Ning, H., Liu, H., Zhang, X., Zhao, Y., & Zhang, S. (2025). Preparation, properties, and applications of eutectogels. *Zeitschrift Für Anorganische Und Allgemeine Chemie*, 651(10). https://doi.org/10.1002/zaac.202500039

Dixon L. (2016, Jun). Will Carrageenan Remain in Organic Food. https://www.cornucopia.org/2016/06/will-carrageenan-remain-organic-food/

Faria, G. S. M. de, Hayashi, L., & Monteiro, A. R. (2014). Effect of drying temperature on carrageenan yield and quality of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) cultivated in Brazil. *Journal of Applied Phycology*, 26(2), 917-922. https://doi.org/10.1007/s10811-013-0172-7

Ferdiansyah, R., Abdassah, M., Zainuddin, A., Rachmaniar, R., & Chaerunisaa, A. Y. (2023). Effects of alkaline solvent type and pH on solid physical properties of carrageenan from *Eucheuma cottonii*. Gels, 9(5), 397. https://doi.org/10.3390/gels9050397

Ferreira, A. S. D., Craveiro, R., Duarte, A. R., Barreiros, S., Cabrita, E. J., & Paiva, A. (2021). Effect of water on the structure and dynamics of choline chloride/glycerol eutectic systems. *Journal of Molecular Liquids*, 342(2021), 117463. https://doi.org/10.1016/j.molliq.2021.117463

Flores, S. L., Descallar, F. B. A., Matsukawa, S., & Bacabac, R. G. (2017). Dynamic rheological properties of mixed carrageenan gels under large strains. *Journal of Biorheology*, 31(2), 35-39. https://doi.org/10.17106/jbr.31.35

Food and Agriculture Organization. (2014). Carrageenan in Compendium of Food Additive Specifications, FAO JECFA Monographs 16, Joint FAO/WHO Expert Committee on Food Additives. United States (US): Food and Agriculture Organization, p. 7-12.

Food Safety Magazine. (2016, Nov). Decision: Carrageenan Banned in Organic Foods. https://www.food-safety.com/articles/5079-decision-carrageenan-banned-in-organic-foods

Heriyanto, H., Kustiningsih, I., & Sari, D. K. (2018). The effect of temperature and time of extraction on the quality of semi refined carrageenan (SRC). In H. Heriyanto (Ed.), The 2nd International Conference on Engineering and Technology for Sustainable Development (ICET4SD 2017) (Vol. 154, pp. 1-5. MATEC Web of Conferences. https://doi.org/10.1051/matecconf/201815401034

Hernanndez-Carmona, G., Freile-Pelegrin, Y., & Hernandez-Garibay, E. (2013). Conventional and Alternative Technologies for The Extraction of Algal Polysaccharides. Woodhead Publishing Series in Food Science, *Technology and Nutrition*. https://doi.org/10.1533/9780857098689.3.475

Horkay, F., & Douglas, J. F. (2018). Polymer Gels: Basics, Challenges, and Perspectives [Chapter]. In ACS Symposium Series (Vol. 1296). *American Chemical Society*. https://doi.org/10.1021/bk-2018-1296.ch001

Hu, R., Sun, D.-W., & Tian, Y. (2025). Emerging eutectogel materials: Development, synthesis, properties, and applications in food science. *Trends in Food Science &*

Technology, 159, 104962. https://doi.org/10.1016/j.tifs.2025.104962

Iijima, M., Hatakeyama, T., & Hatakeyama, H. (2014). Gel-sol-gel transition of kappa-carrageenan and methylcellulose binary systems studied by differential scanning calorimetry. *Thermochimica Acta*, 596(2014), 63-69. https://doi.org/10.1016/j.tca.2014.09.021

Jamaludin, J., Adam, F., Rasid, R. A., & Hassan, Z. (2017). Thermal studies on Arabic gum - carrageenan polysaccharides film. *Chemical Engineering Research Bulletin*, 19(2017), 80-86. https://doi.org/10.3329/cerb.v19i0.33800

Jumaah, F. N., Mobarak, N. N., Ahmad, A., Ghani, M. A., & Rahman, M. Y. A. (2015). Derivative of iota-carrageenan as solid polymer electrolyte. *Ionics*, 21(5), 1311-1320. https://doi.org/10.1007/s11581-014-1306-x

Liu, Y., An, D., Xiao, Q., Chen, F., Zhang, Y., Weng, H., & Xiao, A. (2022). A novel k-carrageenan extracting process with calcium hydroxide and carbon dioxide. *Food Hydrocolloids*, 127(2021), 1-9. https://doi.org/10.1016/j.foodhyd.2022.107507

Liu, Y., Friesen, J. B., Mcalpine, J. B., Lankin, D. C., Chen, S., & Pauli, G. F. (2018). Natural deep eutectic solvents: properties, applications, and perspectives. *Journal of Natural Products*, 81(3), 679-690. https://doi.org/10.1021/acs.jnatprod.7b00945

Mahmood, W. A. K., Khan, M. M. R., & Yee, T. C. (2014). Effects of reaction temperature on the synthesis and thermal properties of carrageenan ester. *Journal of Physical Science*, 25(1), 123-138. https://jps.usm.my/carrageenan-ester/

Martín-del-Campo, A., Fermín-Jiménez, J. A., Fernández-Escamilla, V. V., Escalante-García, Z. Y., Macías-Rodríguez, M. E., & Estrada-Girón, Y. (2021). Improved extraction of carrageenan from red seaweed (*Chondracantus canaliculatus*) using ultrasound-assisted methods and evaluation of the yield, physicochemical properties and functional groups. *Food Science and Biotechnology*, 30(7), 901-910. https://doi.org/10.1007/s10068-021-00935-7

Marullo, S., Meli, A., Giannici, F., & D'Anna, F. (2018). Supramolecular eutecto gels: fully natural soft materials. *ACS Sustainable Chemistry and Engineering*, 6(10), 12598-12602. https://doi.org/10.1021/acssuschemeng.8b04278

Marullo, S., Petta, F., Infurna, G., Dintcheva, N. T., & D'Anna, F. (2023). Polysaccharide-based supramolecular bicomponent eutectogels as sustainable antioxidant materials. *Green Chemistry*, 25(11), 4513-4527. https://doi.org/10.1039/d3gc00573a

Mcreynolds, C., Adrien, A., Castejon, N., & Fernandes, S. C. (2022). Green in the deep blue: deep eutectic solvents as versatile systems for the processing of marine biomass. *Green Chemistry Letters and Reviews*, 15(2), 383-404. https://doi.org/10.1080/17518253.2022.2065890

Nicolau, A., Mutch, A. L., & Thickett, S. C. (2024). Applications of functional polymeric eutectogels. *Macromolecular Rapid Communications*, 2400405, 1-26. https://doi.org/10.1002/marc.202400405

Nishinari, K. (2009). Some thoughts on the definition of a gel. Progress in Colloid and Polymer Science, 136, 87-94. https://doi.org/10.1007/2882_2009_12

Nunez-Santiago, M. del C., & Tecante, A. (2007). Rheological and calorimetric study of the sol-gel transition of k-carrageenan. *Carbohydrate Polymers*, 69(4), 763-773. https://doi.org/10.1016/j.carbpol.2007.02.017

Paiva, A., Craveiro, R., Aroso, I., Martins, M., Reis, R. L., & Duarte, A. R. C. (2014). Natural deep eutectic solvents - solvents for the 21st century. *Journal of American Chemical Society*, 2(5), 1063-1071. https://doi.org/10.1021/sc500096j

Pari, R. F., Uju, U., Ramadhan, W., Hardiningtyas, S. D., Toyofuku, K., Wakabayashi, R., Goto, M., & Kamiya, N. (2025). Deep eutectic solvent-assisted pretreatment of ulva seaweed cellulose and in silico characterization. *Journal of Molecular Liquids*, 435, 128095. https://doi.org/10.1016/j.molliq.2025.128095

Popescu, M. C., Dogaru, B. I., Sun, D., Stoleru, E., & Simionescu, B. C. (2019). Structural and sorption properties of bio-nanocomposite films based on k-carrageenan and cellulose nanocrystals. *International Journal of Biological Macromolecules*, 135(2019), 462-471. https://doi.org/10.1016/j.ijbiomac.2019.05.194

Prasetyaningrum, A., Jos, B., Ariyanti, D., Handayani, N., Rohmah, S. F., Fauzi, H. H., Hakiim, A. A., & Ashianti, A. D. (2024). Effect of concentration of kappa-carrageenan on the physico-chemical properties of rice starch film. *Food Research*, 8(Suppl. 1), 54-58. https://doi.org/10.26656/fr.2017.8(S1).8

Rahman, M. M., & Lamsal, B. P. (2021). Ultrasound-assisted extraction and modification of plant-based proteins: impact on physicochemical, functional, and nutritional properties. *Comprehensive Reviews In Food Science And Food Safety*, 20(2), 1457-1480. https://doi.org/10.1111/1541-4337.12709

Ramadhan, W., Alamsyah, A., Uju, Hardiningtyas, S. D., Pari, R. F., Wakabayashi, R., Kamiya, N., & Goto, M. (2024). Facilitating ulvan extraction from *Ulva lactuca* via deep eutectic solvent and peracetic acid treatment. *ASEAN Journal of Chemical Engineering*, 24(1), 90-101. https://doi.org/10.22146/ajche.12272

Ramadhan, W., Uju, Hardiningtyas, S. D., Pari, R. F., Nurhayati, & Sevica, D. (2022). Ekstraksi polisakarida ulvan dari rumput laut Ulva lactuca berbantu gelombang ultrasonik pada suhu rendah. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 25(1), 132-142. https://doi.org/10.17844/jphpi.v25i1.40407

Ruiz?Olles, J., Slavik, P., Whitelaw, N. K., & Smith, D. K. (2019). Self?assembled gels formed in deep eutectic solvents: supramolecular eutectogels with high ionic conductivity. *Angewandte Chemie*, 131(13), 4217-4222. https://doi.org/10.1002/ange.201810600

Sakti, A. S., Saputri, F. C., & Mun'im, A. (2019). Optimization of choline chloride-glycerol based natural deep eutectic solvent for extraction bioactive substances from Cinnamomum burmannii barks and Caesalpinia sappan heartwoods. *Heliyon*, 5(12), e02915. https://doi.org/10.1016/j.heliyon.2019.e02915

Sedayu, B. B., Cran, M. J., & Bigger, S. W. (2020). Reinforcement of refined and semi-refined carrageenan film with nanocellulose. *Polymers*, 12(5), 1-21. https://doi.org/10.3390/POLYM12051145

Shen, Y.-R., & Kuo, M.-I. (2017). Effects of different carrageenan types on the rheological and water-holding properties of

tofu. LWT - *Food Science and Technology*, 78(2017), 122-128. https://doi.org/10.1016/j.lwt.2016.12.038

Souza, G. de A. L., Pietro, M. E. Di, & Mele, A. (2024). Eutectic solvents and low molecular weight gelators for next-generation supramolecular eutectogels: a sustainable chemistry perspective. *RSC Sustainability*, 2(2), 288-319. https://doi.org/10.1039/d3su00264k

Sun, L., Wang, L., Li, J., & Liu, H. (2014). Characterization and antioxidant activities of degraded polysaccharides from two marine Chrysophyta. *Food Chemistry*, 160(2014), 1-7. https://doi.org/10.1016/j.foodchem.2014.03.067

Torres, M. D., Chenlo, F., & Moreira, R. (2016). Rheology of κ/ι-hybrid carrageenan from Mastocarpus stellatus: critical parameters for the gel formation. *International Journal of Biological Macromolecules*, 86, 418-424. https://doi.org/10.1016/j.ijbiomac.2016.01.096

Tsubaki, S., Oono, K., Hiraoka, M., Onda, A., & Mitani, T. (2016). Microwave-assisted hydrothermal extraction of sulfated polysaccharides from Ulva spp. and Monostroma latissimum. *Food Chemistry*, 210, 311-316. https://doi.org/10.1016/j.foodchem.2016.04.121

Uju, Santoso, J., Ramadhan, W., & Abrory, M. F. (2018). Ekstraksi native agar dari rumput laut Gracilaria sp. dengan akselerasi ultrasonikasi pada suhu rendah. *JPHPI*, 21(3), 414-422. http://dx.doi.org/10.17844/jphpi.v21i3.24711

Ummat, V., Sivagnanam, S. P., Rajauria, G., O'Donnell, C., & Tiwari, B. K. (2021). Advances in pre-treatment techniques and green extraction technologies for bioactives from seaweeds. *Trends in Food Science & Technology*, 110(2021), 90-106. https://doi.org/10.1016/j.tifs.2021.01.018

Wang, J., Zhang, S., Ma, Z., & Yan, L. (2021). Deep eutectic solvents eutectogels: progress and challenges. *Green Chemical Engineering*, 2(4), 359-367. https://doi.org/10.1016/j.gce.2021.06.001

Weerasinghe, U. A., Wu, T., Chee, P. L., Yew, P. Y. M., Lee, H. K., Loh, X. J., & Dan, K. (2024). Deep eutectic solvents towards green polymeric materials. *Royal Society of Chemistry*, 26, 8497-8527. https://doi.org/10.1039/d4gc00532e

Xia, H., Ren, M., Zou, Y., Qin, S., & Zeng, C. (2020). Novel biocompatible polysaccharide-based eutectogels with tunable rheological, thermal, and mechanical properties: The role of water. *Molecules*, 25(15), 3314. https://doi.org/10.3390/molecules25153314

Youssouf, L., Lallemand, L., Giraud, P., Soule, F., Bhaw-Luximon, A., Meilhac, O., D'Hellencourt, C. L., Jhurry, D., & Couprie, J. (2017). Ultrasound-assisted extraction and structural characterization by NMR of alginates and carrageenans from seaweeds. *Carbohydrate Polymers*, 166, 55-63. https://doi.org/10.1016/j.carbpol.2017.01.041

Zeng, C., Zhao, H., Wan, Z., Xiao, Q., Xia, H., & Guo, S. (2020). Highly biodegradable, thermostable eutectogels prepared by gelation of natural deep eutectic solvents using xanthan gum: preparation and characterization. *RSC Advances*, 10(47), 28376-28382. https://doi.org/10.1039/d0ra03390a

Zhao, J., Sun, C., Li, H., Dong, X., & Zhang, X. (2020). Studies on the physicochemical properties, gelling behavior and drug release performance of agar/k-carrageenan mixed hydrogels. *International Journal of Biological Macromolecules*, 154, 878-887. https://doi.org/10.1016/j.ijbiomac.2020.03.087