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# Comparative Characterization of Carrageenan Extracted KOH Treatment and Commercially Available Counterparts

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**ABSTRACT:** The development of seaweed-derived products, particularly carrageenan, is increasingly prioritized in Indonesia to support sustainability and strengthen the local economy. Despite extensive studies on carrageenan extraction, systematic comparisons between locally extracted carrageenan and specific local commercial products remain limited. This study addresses this gap by directly comparing carrageenan extracted from *Eucheuma cottonii* harvested in Lombok, Indonesia, with a locally produced commercial carrageenan as a quality benchmark. Carrageenan extraction was performed using alkaline KOH treatment followed by ethanol precipitation. The extracted carrageenan exhibited a relatively high viscosity (61.16 cP) and a low sulfate content (11.58%). FTIR analysis confirmed the presence of sulfate ester groups and 3,6-anhydrogalactose units, indicating a  $\kappa$ -carrageenan structure. In addition, the extracted carrageenan exhibited a crystallinity index of 31.27% and a gel strength of 539 Pa. The yield, rheological behavior, and total phenolic content (TPC) were also evaluated. Notably, the extracted carrageenan demonstrated structural and functional properties comparable to those of the local commercial product. These findings provide direct evidence that carrageenan derived from Indonesian seaweed meets commercial performance benchmarks, highlighting its potential as a sustainable, competitive raw material for pharmaceutical applications as well as drug delivery.

**KEYWORDS:** Red seaweed; chemical extraction; polysaccharide;  $\kappa$ -carrageenan; semi refined carragenan; industrial-grade comparism

## 1 Introduction

Environmentally friendly and sustainable products are gaining popularity across various industries. Seaweed and its derivatives are widely applied in cosmetics, food products, pharmaceuticals, packaging materials, and edible biomaterials [1]. In recent years, Southeast Asian countries, particularly Indonesia, have become major suppliers of dried red algae and semi-refined carrageenan to the global market [2,3]. According to The State of World Fisheries and Aquaculture (SOFIA) [4], Indonesia is the world leading producer of *Kappaphycus alvarezii* (formerly known as *Eucheuma cottoni*) seaweed, accounting for 82.7% of global production, or approximately 7 million tons in 2021. Seaweed cultivation is widespread across the Indonesian archipelago, especially in Sulawesi, Nusa Tenggara, and East Java [5]. The abundance of *E.*

*cottoni*, along with its ease of cultivation, minimal environmental impact, low production cost, and desirable physicochemical properties, underscores Indonesia strong potential as a major seaweed producer [6]. However, environmental degradation and unsustainable cultivation practices continue to pose significant challenges. Therefore, Indonesia requires a sustainable development strategy that increases seaweed value through downstream processing, such as carrageenan, for applications in the food industry, pharmaceuticals, and packaging [7].

*Kappaphycus alvarezii* (*Eucheuma cottoni*) is a type of red alga that contains high-molecular-weight polysaccharides known as carrageenan. Carrageenan is a hydrophilic, linear polymer composed of repeating disaccharide units, some of which are sulfated. The presence of sulfate ester groups gives carrageenan a negative charge, enabling it to form salt complexes with sodium, potassium, calcium, and magnesium in its natural state [8]. Carrageenan is typically produced by extracting seaweed under alkaline conditions at high temperatures. It is a natural, linear, anionic, sulfated polysaccharide with a molecular weight ranging from approximately 100–1000 kDa, consisting of repeating units of D-galactose and 3,6-anhydrogalactose with  $\alpha$ -1,3 and  $\beta$ -1,4 glycosidic bonds [9]. Carrageenan is classified into six types: kappa ( $\kappa$ ), iota ( $\iota$ ), lambda ( $\lambda$ ), tetha ( $\theta$ ), mu ( $\mu$ ), and nu ( $\nu$ ) [10,11]. Carrageenan is widely used in various industries, particularly in the food sector, where it functions as a stabiliser, thickener, emulsifier, and gelling agent. In the pharmaceutical field, it is used in wound healing, tissue engineering, and capsule production. Over the past 15 years, the carrageenan industry has grown at an annual average rate of 8% per year, reaching a production volume of 28.000 metric tons [12,13]. Encouraging Indonesia, as one of the major seaweed producers, to develop carrageenan-based products to increase market value and meet consumer demand.

The quality of carrageenan extracted from seaweed is influenced by several factors, including the origin of the raw material, the extraction method, processing conditions, and the sulfate content in carrageenan. Previous studies reported that carrageenan from Indian waters has an average yield of approximately 50% with a gel strength of 379 g·cm<sup>-2</sup> [13]. In comparison, carrageenan from Indonesia waters has a yield ranging from 17%–56%, with a gel strength of 897 g·cm<sup>-2</sup>, and a viscosity of 76.15 cP [14]. Carrageenan from Malaysian waters has been reported to exhibit an average yield of 65.5%, a gel strength of 1.456 g·cm<sup>-2</sup>, and a viscosity of 58 cP [15,16]. In addition to geographical factors, the extraction method also significantly influences the final quality of carrageenan. Carrageenan extracted using a conventional high-temperature alkali treatment, followed by alcohol precipitation, generally shows superior characteristics, with a recovery yield of 77.33% and a viscosity of 50.87 cP [17]. These values are higher than those obtained through enzymatic extraction (yield 27.6%; gel strength 11.6 g·cm<sup>-2</sup>) or microwave-assisted methods (yield 17.16%; viscosity 21 cP; gel strength 26 g·cm<sup>-2</sup>) [18]. The use of alcohol for precipitation is commonly applied in carrageenan gel formation as an alternative to KCl or CaCl<sub>2</sub>. Alcohol is particularly effective for the purification of  $\kappa$ -carrageenan. Alcohol precipitation produces the highest purity and most refined product, and it is utilized in pharmaceuticals, toothpaste, and high-value food products such as jams, jellies, soups, and ice cream. Approximately 19% of the global carrageenan market is produced using alcohol precipitation, and it is expected to grow at a rate of 2.9% annually in volume until 2030 [19]. Refined carrageenan requires further filtration and purification steps to remove components such as cellulose, salt, dissolved sugars, proteins, and other impurities [20]. During alkali extraction, the sulfate content can be significantly reduced, while the 3,6-anhydrogalactose content increases, resulting in higher-quality and improved functional carrageenan [21]. Collectively, these factors affect the structure and conformation of the carrageenan polymer chain, which ultimately impacts the yield, sulfate content, viscosity, gel strength, and crystallinity [22].

Several previous studies have reported the extraction of carrageenan. However, most studies have focused on optimizing the extraction process rather than conducting a systematic comparison between

extracted carrageenan and commercial products that serve as established quality benchmarks. Such comparative evaluations are important, particularly because local carrageenan from Lombok, Indonesia, has the potential as an alternative industrial raw material, especially in major seaweed-producing regions such as West Nusa Tenggara. Assessing the physicochemical properties, structure features, and gel-forming behavior of the extracted carrageenan is crucial to determine whether it exhibits performance comparable to, or exceeding, that of commercial carrageenan and whether it conforms to the Indonesian National Standards (SNI). Building upon this rationale, the present study aims to compare the characteristics of carrageenan extracted from Lombok, Indonesia, using an alkali (0.5 N KOH at 75°C, 60 min) method, followed by an alcohol precipitation method, with those of commercial carrageenan through a comprehensive analysis of structural, physicochemical, and functional properties. The assessment includes measurements of sulfate content, viscosity, gel strength, chemical structure, crystallinity, and total phenolic content. This systematic comparison is expected to provide deeper insights into the quality of the extracted carrageenan and to offer a direct comparison with a specific local commercial product, highlighting its potential suitability for various local industrial applications, including packaging, food, and pharmaceutical products.

## 2 Materials and Methods

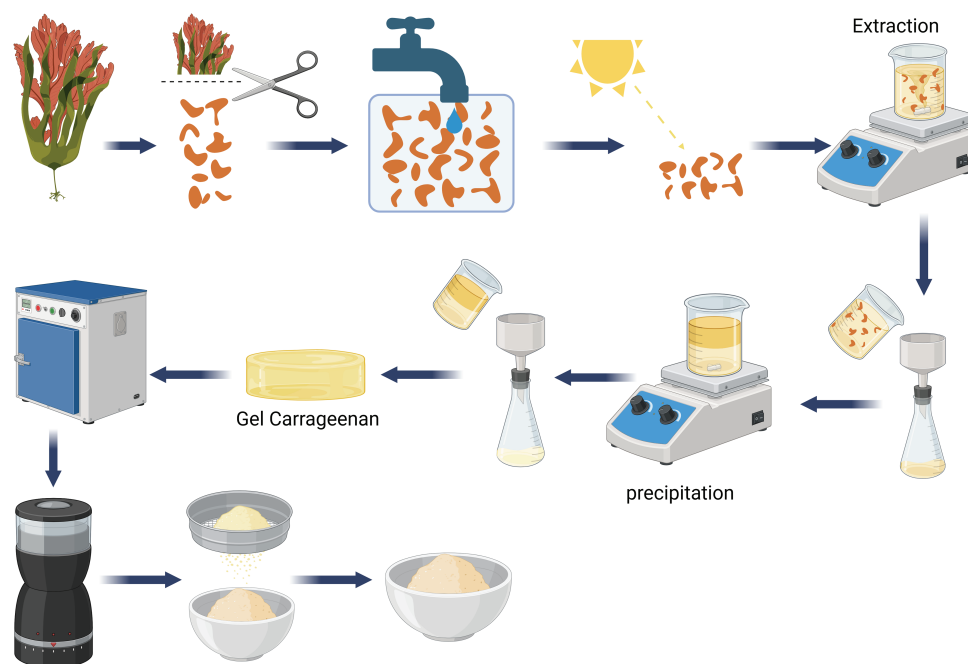
### 2.1 Materials

*Echeuma cottoni* seaweed was obtained from Lombok, West Nusa Tenggara, Indonesia. Potassium hydroxide (KOH) pellets, hydrochloric acid (HCl, 37%), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%), and barium chloride (BaCl<sub>2</sub>) were purchased from Merck (Germany). Technical-grade ethanol (96%) and commercial carrageenan were obtained from PT. Indochem Indonesia is used as a reference material. The commercial carrageenan had a water-gel strength of 1299 g/cm<sup>3</sup> and a particle size of 85% passing through a 60-mesh sieve.

### 2.2 Methods

The preparation material began with dried seaweed, which was cut into approximately 1 cm segments and thoroughly washed with distilled water to remove adhering sand and impurities. The cleaned seaweed was sun-dried until the moisture content reached approximately 10%.

The dried seaweed was initially soaked in distilled water for 15 min. The soaked seaweed was subsequently extracted at a solid-to-liquid ratio of 1:50 (w/v) with 0.5 N KOH solution. The extraction process was conducted for 60 min at 75°C under continuous stirring. After extraction, the mixture was filtered using a 500-mesh filter cloth to separate the filtrate from the residue. The resulting filtrate was then precipitated by gradual addition of cold ethanol (5°C) at a 1:3 (v/v) ratio to induce polysaccharide precipitation. The precipitation process was carried out for 30 min with continuous stirring. The precipitation gel was subsequently filtered again using a 500-mesh filter cloth and dried in a hot-air oven at 60°C for 24 h. Finally, the dried carrageenan was ground and sieved to obtain particles smaller than 200 mesh. The process of extraction is illustrated in [Fig. 1](#).



**Figure 1:** Extraction of carrageenan from *Kappaphycus alvarezii*.

## 2.3 Characterization

### 2.3.1 Yield

The extraction yield was calculated based on the ratio of the mass of carrageenan obtained to the mass of dried seaweed used. The yield was determined using Eq. (1).

$$\text{Yield (\%)} = \frac{\text{mass of carrageenan extract (g)}}{\text{mass of dried seaweed (g)}} \times 100\% \quad (1)$$

### 2.3.2 Sulfate Content

One gram of the carrageenan sample was placed in an Erlenmeyer flask, and 50 mL of 0.2 N HCl was added. The mixture was heated for 1 h, followed by the addition of 25 mL of 10% (v/v) H<sub>2</sub>O<sub>2</sub>, and then reheated for 5 h or until the solution became clear. After clarification, 10 mL of 10% (w/v) BaCl<sub>2</sub> was added dropwise, and the mixture was reheated for an additional 2 h. The resulting precipitate was filtered using ash-free filter paper (Whatman No. 42) and washed thoroughly with hot distilled water. The paper containing the precipitate was then incinerated in a furnace at 1000°C until it was completely ashed. After cooling in a desiccator, the residue was weighed. The sulfate content was calculated using Eq. (2).

$$\text{Sulfate content (\%)} = \left( \frac{\text{Mass of BaSO}_4 \text{ precipitate (g)} \times 0.4116}{\text{Mass of Carrageenan (g)}} \times 100\% \right) \quad (2)$$

### 2.3.3 Viscosity and Gel Strength

A 1.5% (v/v) carrageenan solution was heated to 80°C under constant stirring. The viscosity was measured at 75°C using a Brookfield viscometer equipped with spindle No. 62 at a speed of 60 rpm. For the gel strength analysis, the carrageenan solution was transferred into a mold and allowed to set, after which the gel strength was analyzed using a rheometer by Rhein-Knudsen et al. method [23].

### 2.3.4 Fourier Transform Infrared (FTIR) Analysis

The functional groups were analyzed using a PerkinElmer Japan Co., Ltd. UATR-FTIR Spectrometer. The spectra were recorded with four scans at a resolution of  $4\text{ cm}^{-1}$  over the wavenumber range of  $4000\text{--}400\text{ cm}^{-1}$ .

### 2.3.5 X-Ray Diffraction (XRD) Analysis

The crystallinity index of carrageenan was analyzed using an X'Pert PRO PAN diffractometer (Philips Analytical, Netherlands) equipped with Cu K $\alpha$  radiation ( $\lambda = 0.154\text{ nm}$ ), operated at 40 kV and 30 mA. The diffraction patterns were recorded over a scanning range of  $5^\circ$  to  $50^\circ$  ( $2\theta$ ). The crystallinity index (CI) was calculated using the Segal et al. [24] Method, as shown in Eq. (3).

$$CI = \left[ \frac{I_{002} - I_{am}}{I_{002}} \right] \times 100\% \quad (3)$$

where  $I_{002}$  is the diffraction intensity at  $2\theta = 21^\circ\text{--}24^\circ$ , representing the crystalline region, and  $I_{am}$  is the intensity at  $2\theta = 18^\circ$ , representing the amorphous region.

### 2.3.6 Total Phenolic Content (TPC)

The total phenolic content (TPC) of carrageenan was determined using the Folin-Ciocalteu method. Dried extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain a 20 mg/mL solution. In a microcentrifuge tube, 600  $\mu\text{L}$  of distilled water, 10  $\mu\text{L}$  of sample, and 50  $\mu\text{L}$  of Folin-Ciocalteu reagent were mixed. After 1 min, 150  $\mu\text{L}$  of 20% (w/v)  $\text{Na}_2\text{CO}_3$  and 190  $\mu\text{L}$  of distilled water were added. Subsequently, 300  $\mu\text{L}$  of the homogenized mixture was transferred into a 96-well microplate. After incubation for 2 h in the dark at room temperature, the absorbance was measured at 760 nm in a microplate reader (Tecan Infinite M200). The analyses were performed at least in triplicate. TPC values were calculated using a gallic acid calibration curve ( $125\text{--}1000\text{ }\mu\text{g/mL}$ ) and expressed as mg of Gallic Acid Equivalent per gram of extract ( $\text{mg of GAE}\cdot\text{g}^{-1}$ ).

### 2.3.7 Statistical Analysis

The data were repeated in triplicate, and the results are presented as means and standard deviations. Analysis of variance (ANOVA) was used to determine significant differences, and the Duncan test was applied using the SPSS analysis application. Values of  $p < 0.05$  were considered statistically significant.

## 3 Result and Discussion

The result of the data analysis and comparison of the characterization of carrageenan extraction in this study including yield, viscosity, and sulfate content, are shown in Table 1 below.

**Table 1:** Characterization of Carrageenan.

Extraction Method	Yield (%)	Viscosity (cP)	Sulfate Content (%)	Type of Carrageenan
KOH	$18.31 \pm 1.05$	$61.16 \pm 0.07$	$11.58 \pm 1.55$	This study (carrageenan extraction)
–	–	52.83	$1.23 \pm 1.54$	This study (carrageenan commercial)
MAE-Water	17.16	21.00	–	[18]

(Continued)

**Table 1 (continued)**

Extraction Method	Yield (%)	Viscosity (cP)	Sulfate Content (%)	Type of Carrageenan
Phosphate Buffer	28.70–44.00	–	16.20–39.90	[25]
Ca(OH) <sub>2</sub>	36.20	160.00	18.72	[22]
KOH	37.02	–	11.45	[26]
Ca(OH) <sub>2</sub>	24.70	25.40	26.70	[27]
Water	68.00	120.00	9.44	[28]
KOH	25.69–30.65	1.13–5.93	18.24–20.64	[29]
Quality Standart	>25	>5	15–40	[30]

### 3.1 Yield

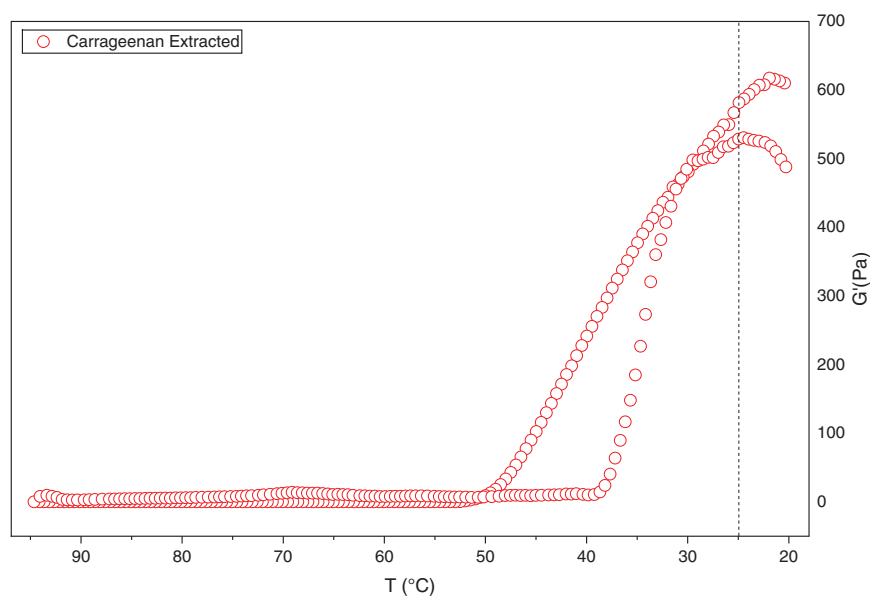
As presented in Table 1, the yield of extracted carrageenan in this study was  $18.31 \pm 1.05\%$ , which is relatively lower than that reported in a previous study using KOH and Ca(OH)<sub>2</sub>, which achieved yields of 37.2% and 24.7%, respectively, and is also below the commonly accepted quality standard (>25%). This relatively low yield is primarily due to the degradation of carrageenan polymer chains during alkaline extraction, resulting in a reduction in molecular weight. Alkaline treatment, particularly when combined with elevated temperatures, can induce depolymerization of polysaccharide chains, resulting in a lower molecular weight of carrageenan. Consequently, during the precipitation process, the low molecular weight of carrageenan fractions exhibits poor recovery efficiency, thereby contributing to the reduced yield. This phenomenon is consistent with the previous studies reporting that polysaccharide degradation occurs in seaweed during the cooking and alkali extraction process [31]. In addition, during the extraction, proteins, carbohydrates, and soluble salts are simultaneously lost along with the extraction solution, which further decreases mass recovery and ultimately reduces the carrageenan yield [30].

In addition to process parameters such as alkali solution concentration, extraction temperature, and extraction time, the type of seaweed, cultivation source, climate, and growth environment (including salinity, temperature, water movement, depth, and nutrient availability) also significantly influence carrageenan yield [13]. The carrageenan yield of *K. alvarezii* cultivated for 42 days can exceed 28%, whereas a longer cultivation period of 97 days results in a lower yield of approximately 25% [32,33]. This reduction is attributed to the transition from the vegetative to the generative phase, during which the carrageenan content decreases despite continued biomass growth, reaching its maximum [34]. Seaweed adsorbs essential nutrients such as carbon, hydrogen, oxygen, sulfate, and nitrate, which are subsequently converted through photosynthesis into polysaccharides in the form of carrageenan and stored in the cell walls. Any disruption in the photosynthesis process inhibits seaweed growth and reduces carrageenan production [35]. Furthermore, several seaweed species cultivated in Indonesian waters have been reported to exhibit varying carrageenan yields, including 7.73% in Makassar waters [36], 16.8% in Banyuwangi waters [37], and 18.42% at Banggai beach [29]. These values are comparable to the yield obtained in this study using seaweed from Lombok. Therefore, the carrageenan extraction method employing KOH treatment followed by ethanol precipitation, as demonstrated in this research, shows strong potential for producing carrageenan suitable for the local Indonesian carrageenan industry.

### 3.2 Rheological Properties

Rheological properties were measured by oscillatory rheology. The storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were measured over a temperature range of 95°C–20°C. To evaluate the reversible gelation properties, gelation testing was performed through a heating–cooling cycle within the same temperature range. As shown in Fig. 2, the storage modulus ( $G'$ ) increased significantly with decreasing temperature, indicating the formation of a gel. This increase in  $G'$  reflects the sol–gel transition in the extracted carrageenan. The gelation temperature ( $T_{\text{gel}}$ ) and melting temperature ( $T_{\text{melt}}$ ) were determined as the points at which the storage modulus ( $G'$ ) exceeds the loss modulus ( $G''$ ) during repeated cooling and heating cycles. The extracted carrageenan exhibited a  $T_{\text{gel}}$  of 26°C and a  $T_{\text{melt}}$  of 58°C. The  $T_{\text{gel}}$  value was lower than that of commercial  $\kappa$ -carrageenan, whereas the  $T_{\text{melt}}$  value was comparable to those reported in the literature, including  $\kappa$ -carrageenan from Sigma-Aldrich [23]. These results were consistent, with  $T_{\text{gel}}$  reported as kappa iota hybrid carrageenan at 28.5°C and  $T_{\text{melt}}$  at 52°C, extracted with alkali [34], and were consistent with carrageenan extracted from Vietnam at 23°C [38]. In contrast, in some previous studies, the average  $T_{\text{gel}}$  carrageenan ranged from 28°C to 45°C [23,39]. These findings suggest that distinct factors influence the mechanisms governing the initiation of gelation and the thermal stability of the gel network. The lower gelation temperature suggests that  $\kappa$ -carrageenan helix association occurs at reduced temperatures. Although the sulfate content in this study was relatively low, the results indicate that the gelation temperature is not primarily determined by sulfate content [21]. Instead, the limited availability of counterions, particularly  $K^+$ ,  $Na^+$ , or  $Ca^+$  ions, which play a crucial role in stabilizing the junction zones between helices, is likely the dominant factor. Insufficient  $K^+$  ions hinder the formation of stable double helices at higher temperatures, resulting in gelation only when the thermal energy of the system is sufficiently low to allow chain association [40]. In addition to the ionic effect, the gelation temperature may also be influenced by the characteristics of the polysaccharide chain [41]. Partial degradation during extraction or thermal treatment can reduce molecular weight and shorten chain length, thereby lowering the thermal energy required for the conformational transition to the double-helix structure. Nevertheless, once the gel network is established, the remaining inter-helix interactions are sufficiently strong to maintain the gel thermal stability, as evidenced by the  $T_{\text{melt}}$  value, which does not differ significantly from that in the previous study. The substantial difference between  $T_{\text{gel}}$  and  $T_{\text{melt}}$  further indicates the presence of thermal hysteresis, a characteristic feature of  $\kappa$ -carrageenan systems. This hysteresis confirms that gel formation is susceptible to the initial system conditions, such as ionic composition and molecular weight. In contrast, the melting temperature more accurately reflects the strength and stability of the gel network.

Carrageenan gel strength measurements were conducted at 25°C using the average storage modulus ( $G'$ ) at a frequency of 1 Hz. The gel strength value of carrageenan extracted in this study was 549 Pa, which was relatively close to that of hybrid kappa-iota carrageenan at 689 Pa [42], although it remains lower than values reported in several other studies. This relatively lower gel strength is likely influenced not only by the extraction method used, but also related to the ratio of glucose to galactose units and the relatively uniform sulfate content [43,44]. In addition to these factors, previous studies have reported that gel strength is also affected by molecular weight, ion content, and other structural parameters. These findings are in line with the low yield of carrageenan extracted in this study, which suggests that partial degradation of the polysaccharide chain occurred during extraction. Statistical analysis of the gel strength data, using a one-way ANOVA followed by the Duncan multiple-range test, revealed a significant difference ( $p < 0.05$ ).



**Figure 2:** Oscillatory rheological carrageenan, Storage modulus  $G'$  (Pa), measured from 95°C to 20°C at a rate of 1°C/min.

### 3.3 Viscosity

The viscosity of the carrageenan extracted was slightly higher than that of the commercial carrageenan, with values of  $61.16 \pm 0.07$  cP and  $52.83 \pm 0.02$  cP, respectively. The statistician is involved in one-way analysis of variance (ANOVA) and the Duncan multiple-comparison post hoc test at the 95% confidence level among the applied treatments ( $p < 0.05$ ). This viscosity was also markedly higher than that obtained with MAE-water extraction (21 cP) [18]. This increase can be attributed to the effect of KOH alkalization on the chemical structure of carrageenan, which enhances its viscosity. Ferdiansyah et al. reported a similar phenomenon, in which KOH extraction converted sulfate groups into 3,6-anhydrogalactose, thereby improving the ability of carrageenan to form gel networks and double helix structure [29]. These molecular chains subsequently become more rigid and form flow-inhibiting aggregates, leading to an increase in viscosity. These findings are consistent with previous reports indicating that carrageenan viscosity is closely related to its sulfate content. The negatively charged sulfate groups induce electrostatic repulsion between polymer chains, increasing chain rigidity and, consequently, the viscosity of the system [40]. The results of this study align with previous research, which also found that carrageenan extraction using alkali followed by alcohol precipitation increases viscosity values, reaching 50 cP, compared to enzymatic extraction [30].

### 3.4 Sulfate Content

Seaweed containing sulfated polysaccharides such as carrageenan ( $\text{SO}_3^-$ ) plays an important role in the prevention, acceleration, and regulation of wound healing processes. In this study, the extracted carrageenan exhibited a higher sulfate content than the commercial carrageenan, with values of  $11.58\% \pm 1.55\%$  and  $1.23\% \pm 1.55\%$ , respectively. The statistician is involved in one-way analysis of variance (ANOVA) and the Duncan multiple-comparison post hoc test at the 95% confidence level among the applied treatments ( $p < 0.05$ ). This difference is attributed to the alkaline treatment during extraction, which alters the sulfate groups within the galactose units, leading to changes in the overall sulfate content of carrageenan. The hydroxide ions from the alkaline reagent penetrate the seaweed tissue, promoting partial desulfation while simultaneously increasing the formation of 3,6-anhydrogalactose. In parallel, potassium ions interact with

carrageenan chains, forming a gel network that stabilizes carrageenan at elevated temperatures and prevents melting in hot solutions. However, despite the increase compared to commercial carrageenan, the sulfate content of the extracted carrageenan remains below the FAO standard of 15%–40% [45] and is comparable to that reported in a previous study using KOH extraction at 11.45% [46].

The sulfate content of carrageenan obtained in this study was found to be below the Food and Agriculture Organization (FAO) standard, in contrast to several previous reports that documented sulfate levels within the recommended range. This difference is likely associated with variations in the purification strategy, particularly the absence of repeated precipitation steps, which may have led to a lower degree of carrageenan purification. As a consequence, the development of the gel network appears limited [47], as reflected by the relatively low gel strength and the reduced gelation temperature observed in this study. This interpretation is supported by rheological measurements, which showed a comparatively low storage modulus ( $G'$ ) and a limited distinction between the storage modulus ( $G'$ ) and loss modulus ( $G''$ ) over the tested temperature range [48]. Such behavior is characteristic of a weak or soft gel structure with restricted elastic dominance. Furthermore, FTIR analysis confirmed the presence of characteristic absorption bands corresponding to S=O stretching vibrations of sulfate ester groups, as well as C–O–S bond vibrations associated with sulfated galactose and 3,6-anhydrogalactose units [49]. While these spectral features indicate that the fundamental carrageenan backbone remains intact, the relatively high transmittance suggests a low abundance of sulfate groups, consistent with the measured sulfate content being below the FAO standard. In contrast, the commercial carrageenan reference did not exhibit well-defined transmittance peaks in the same spectral regions, suggesting differences in chemical structure and degree of sulfation that may arise from distinct production and purification processes. Such structural variations are expected to influence gelling behavior. From an application perspective, the lower degree of sulfation and the formation of a softer gel network observed in this study should not necessarily be considered a drawback for non-food uses. Instead, these characteristics may be advantageous for pharmaceutical, biomedical, hydrogel, and drug delivery applications, where mild gelation conditions, mechanical compliance, and facilitated molecular diffusion are often desirable.

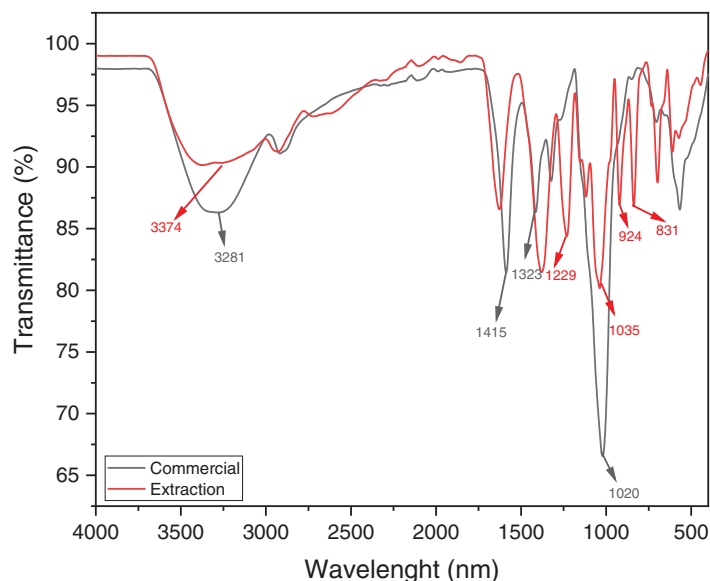
### 3.5 Fourier Transferred Infrared (FTIR)

Fig. 3 and Table 2 display the FTIR spectrum used to identify the functional groups that constitute carrageenan. FTIR analysis is performed by irradiating molecules with infrared light, which induces characteristic vibrational responses that reflect their chemical structure. Because each infrared frequency interacts differently with the material, allowing the composition and structure of the macromolecules to be accurately identified.

The extracted carrageenan powder was subsequently analyzed using FTIR to determine its chemical functional groups. In both extracted and commercial carrageenan, Transmittance peaks were observed at wavenumbers of 3327 and 3281  $\text{cm}^{-1}$ , corresponding to the hydroxyl (–OH) groups, which indicate the hydrophilic nature of carrageenan [50]. A peak at 1229  $\text{cm}^{-1}$  indicates the presence of sulfate ester groups, which are typically formed under alkaline conditions during the extraction process [17]. This band is characteristic of carrageenan. In addition, standard carrageenan bands were also detected at 1035 and 1006  $\text{cm}^{-1}$  for extracted carrageenan, and at 1020  $\text{cm}^{-1}$  for commercial carrageenan. These peaks indicate the presence of glycosidic groups and polysaccharide structures that constitute carrageenan [51]. The presence of a band at 924  $\text{cm}^{-1}$  in extracted carrageenan corresponds to 3,6-anhydrogalactose, while the band at 831  $\text{cm}^{-1}$  indicates D-galactose-4-sulfate, a key structural feature of  $\kappa$ -carrageenan [52]. Stretching vibrations of the anhydroglucose ring were also observed at 575  $\text{cm}^{-1}$ , which have been associated with the antioxidant properties of carrageenan [53]. A weak band at approximately 700  $\text{cm}^{-1}$  in extracted carrageenan

is attributed to skeleton bending of the galactose ring within the carrageenan structure [23]. Furthermore, the transmittance bands at 1154 and 1120  $\text{cm}^{-1}$  correspond to C–O and C–C stretching vibrations of the pyranose ring, which were commonly found in polysaccharides [54]. The bands in the range of 1154  $\text{cm}^{-1}$  and 1020  $\text{cm}^{-1}$  both represent C–O bonds from the pyranosyl ring, while the difference in peak position is due to variations in the proportion of G and M units that comprising the carrageenan structure [54]. In addition to the characteristic carrageenan bands, a peak at 1622  $\text{cm}^{-1}$  observed in extracted carrageenan is associated with the amide I group, indicating the presence of  $\text{H}_2\text{O}$  or CO–NH (amide) groups that likely originate from residual protein components [55].

In commercial carrageenan, no distinct transmittance peak was observed around 1200  $\text{cm}^{-1}$ , suggesting a relatively low sulfate content or levels below the detection limit of infrared spectroscopy. This observation is consistent with the sulfate analysis, which revealed that commercial carrageenan generally has a lower sulfate content than the extracted carrageenan in this study. A similar phenomenon was observed in the 900–800  $\text{cm}^{-1}$  region, where no characteristic transmittance bands were detected in commercial carrageenan, even though this range is commonly used to confirm the type of carrageenan produced. However, in commercial carrageenan, a peak at 1415  $\text{cm}^{-1}$  was observed, which is associated with the carboxymethyl group ( $-\text{CH}_2$ ) and may influence the gel strength of carrageenan [17]. The carboxyl group in the extracted carrageenan was also detected at approximately 1372  $\text{cm}^{-1}$ , with a transmittance intensity comparable to that of commercial carrageenan, namely 81.13% for the extracted carrageenan and 85.97% for commercial carrageenan.



**Figure 3:** FT-IR spectra of carrageenan extraction and carrageenan commercial.

**Table 2:** Spectra bond FT-IR Carrageenan.

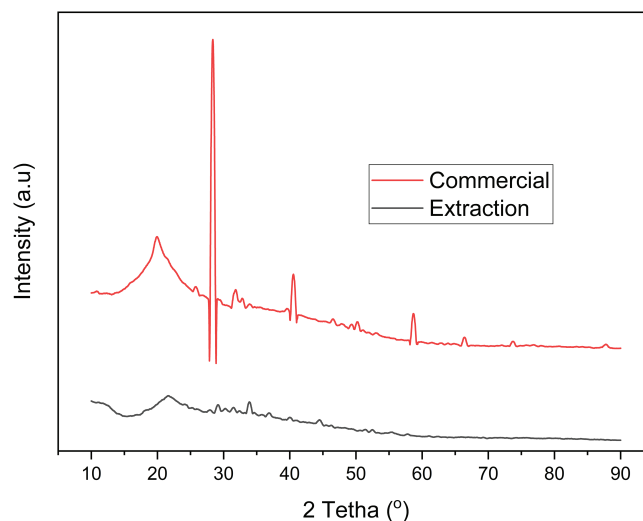
Wavelength Number (cm <sup>-1</sup> )		Band	Ref.
Carrageenan Extraction	Carrageenan Commercial		
3374	3281	-OH Hydroxyl group	[50]
1622		C=O	[56]
1372	1323; 1415	-CH <sub>2</sub> Carboxymethyl group	[31]
-	1586	-CH <sub>2</sub>	
1229		S=O Sulfate ester group	[17]
1154; 1120		C-O pyranonysyl ring	[54]
1035; 1006	1020	C-O of 3,6 anhydrogalactose	[51]
924	-	3,6-anhydrous-D-galactose	[53]
831	-	C-O-SO <sub>3</sub> on D-galactose 4-sulfate	[52]
700		Galactose ring	[23]
575	562	Anhydro-glucose ring	[53]

### 3.6 X-Ray Diffractogram (XRD)

Fig. 4 shows the XRD diffractogram of carrageenan extracted from XRD and commercial sources. The principle of XRD is that X-rays have wavelengths between 0.01 and 10 nm, comparable to the distance between atoms in crystals. When X-rays penetrate crystals, they induce vibrations in the electrons within them, which can be interpreted to determine the crystal structure of a material [57]. In this study, X-ray diffraction analysis was employed to determine the crystal structure and relative crystallinity of the extracted carrageenan and commercial carrageenan. It can be seen that the extracted sample displays characteristic diffraction peaks at  $2\theta = 27.92^\circ, 29.13^\circ, 30.31^\circ, 31.56^\circ, 33.89^\circ, 36.84^\circ, 40.01^\circ, 44.46^\circ, 46.26^\circ, 51.39^\circ,$  and  $52.45^\circ$ , which is a common lattice pattern for polysaccharides. The diffraction patterns of the extracted and commercial polysaccharides exhibit some similarities; however, the commercial carrageenan displays characteristic peaks at approximately  $2\theta = 19.86^\circ, 61.17^\circ, 73.72^\circ,$  and  $87.71^\circ$ . The crystallinity index (CrI) was calculated using the Segal et al. [24] method, with results of 31.27% and 8.07% for the extracted and commercial carrageenan, respectively. The increase in CrI after alkalization is associated with the loss of amorphous structure, resulting in demineralization and deproteinization [58].

### 3.7 Total Phenolics Content (TPC)

The phenolic content of extracted carrageenan is higher than that of commercial carrageenan, at 0.239 mg GAE.g<sup>-1</sup> and 0.125 mg GAE.g<sup>-1</sup>, respectively. The statistician is involved in one-way analysis of variance (ANOVA) and the Duncan multiple-comparison post hoc test at the 95% confidence level among the applied treatments ( $p < 0.05$ ). Extracted carrageenan uses a shorter extraction time, thereby retaining phenolic compounds. Commercial carrageenan, on the other hand, requires a longer extraction time, allowing for the degradation of phenolic compounds during the early stages of extraction. The commercial carrageenan extraction process involves two stages of mass transfer. The first stage is controlled by solute solubility, followed by diffusion. The second stage requires diffusion of compounds dissolved during extraction to the surface of the solid matrix [59,60]. Carrageenan extraction using ethanol, however, produced a higher TPC value of 3.42 mg GAE.g<sup>-1</sup>. Another study found that carrageenan extracted from other types of red seaweed using a similar method produced a TPC of around 0.1–1.1 mg GAE.g<sup>-1</sup> [25].



**Figure 4:** Diffractogram XRD carrageenan extraction and carrageenan commercial.

#### 4 Conclusion

This study shows that carrageenan extracted through an alkalization method followed by a precipitation process produces material with characteristics comparable to commercial carrageenan, thus having the potential to be developed for non-food industrial applications. The yield obtained (18.31%) is within an acceptable range for natural biomass-based extraction systems and is thought to be influenced by intrinsic factors of the raw material, particularly the source and cultivation conditions of the seaweed. This yield variability reflects differences in the structural composition of polysaccharides due to environmental and physiological factors, and does not indicate degradation or ineffectiveness of the extraction process applied. The extracted carrageenan exhibits a relatively low sulfate content (11.58%), which is an important characteristic for biomedical applications and drug delivery systems, as it contributes to the reduction of excess negative charge and supports more controlled diffusion of active molecules. This is reinforced by a fairly high viscosity value (61.16 cP), which indicates the ability to form stable polymer networks, as well as a total phenolic content (TPC) of 0.239 mg GAE g<sup>-1</sup> that has the potential to provide additional functions to the material. FTIR analysis confirmed that the carrageenan obtained is classified as  $\kappa$ -carrageenan. Overall, the combination of structural, rheological, and chemical properties indicates that the produced carrageenan has promising potential for non-food applications, particularly in the fields of drug delivery and biomedicine, including the development of hydrogels, wound dressings, and capsule shells.

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**Author Contributions:** Manda Vais Jatul Fitri—conceptualization, methodology, investigation, data curation, writing—original draft preparation; Yuni Kusumastuti—validation, formal analysis, supervision; Melbi Mahardika—conceptualization, validation, writing—review and editing, supervision, project administration, funding acquisition; Mochamad Asrofi—resources. All authors reviewed and approved the final version of the manuscript.

**Availability of Data and Materials:** The data supporting this study's findings are available from the Corresponding Author, Melbi Mahardika, upon reasonable request.

**Ethics Approval:** Not applicable.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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