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Carrageenan Production and Gel Quality: Interspecific Responses of Eucheumatoid Algae to Extraction Method and Low-Salinity Stress

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Abstract

Low-salt stress caused by rainfall and freshwater runoff severely restricts the large-scale cultivation of the tropical algae Eucheumatoid. In this study, five commercial Eucheumatoid species, including *Kappaphycus alvarezii* (Hainan green and red strains, Malaysia red strain), Malaysia *Kappaphycus striatum* and Hainan *Eucheuma denticulatum*, were selected as the experimental materials. We compared the differences in carrageenan yield, physicochemical properties, and five textural parameters of carrageenan extracted by alkaline extraction and ultrasound-assisted enzymatic extraction methods, as well as those under a low-salinity gradient. Among the five algal species assessed with two extraction methods, Hn-E showed the best comprehensive carrageenan performance (gel yield = 45%; gel strength = 210 g·cm^{-2}, sulfate content = 32%, viscosity > 250 mPa·s; TPA: hardness > 150 g, cohesiveness = 0.73, chewiness = 122.5 g, springiness > 0.88, resilience = 0.72). Enzyme-assisted extraction produced the poorest overall indices for Hn-G and Hn-R. Under low-salinity gradients, 18‰-cultured Ma-R achieved the highest yield (44.5%) and sulfate content (36%). Viscosity increased with salinity without significant inter-species divergence at the same salinity. Gel strength and textural parameters peaked at 24‰, moderately dropped at 32‰ and minimized at 18‰; at each salinity level, these parameters followed the descending order: Hn-E > Ma-R > Ma-Ks > Hn-R > Hn-G. The result of Fourier-transform infrared spectra revealed κ-type carrageenan signatures in all samples and absence of λ-type signals; the dominant ι-type peak was found in alkali-extracted carrageenan of Hn-R and enzyme-assisted extracted carrageenan of Hn-E. This study demonstrates that salinity fluctuation and extraction strategy jointly determine the quality and type of carrageenan, providing references for stabilizing carrageenan quality under low-salinity disturbance and a foundation for germplasm screening and extraction protocol optimization of Eucheumatoid aquaculture in rainfall-prone coastal regions.

Keywords: Eucheumatoids; carrageenan; low-salt stress; gel properties; textural parameters

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1. Introduction

Eucheumatoid belongs to the taxonomies Rhodophyta, Rhodophyceae, Gigartinales and Solieriaceae, and comprises *Kappaphycus* Doty, *Eucheuma* J. Agardh, and *Betaphycus* Doty [1]. The three genera of Eucheumatoid supply the vast majority of raw materials

for carrageenan production and represent the most economically important carrageenan-producing seaweeds worldwide [2]. The core distinction among the three genera lies in the type of carrageenan they synthesize. *Kappaphycus striatus*, *Eucheuma denticulatum* and *Kappaphycus alvarezii* within Eucheumatoid have been extensively cultivated on a large scale and serve as the predominant raw materials for commercial carrageenan production [3].

Carrageenan is a high-molecular-weight sulfated polysaccharide distributed in the cell walls and intercellular matrix of macro red algae [4]. Carrageenans can be classified into six primary types based on the presence or absence of 3,6-anhydrogalactose in the repeating units of the linear backbone, as well as the content and molecular linkage position of sulfate groups: kappa-(κ), iota-(ι), lambda-(λ), mu-(μ), nu-(ν), and theta-(θ) [5]. The three most common types are κ-carrageenan, with one sulfate group, ι-carrageenan, with two sulfate groups, and λ-carrageenan, with three sulfate groups [6].

Algae of the genus *Kappaphycus*, mainly represented by *K. alvarezii* and *K. striatus*, are the principal source of κ-carrageenan [7]. As it has the most favorable water- and oil-retention properties, κ-carrageenan is widely applied in the food, pharmaceutical and cosmetic industries, ranking as the most in-demand and extensively utilized type of carrageenan [8]. The genus *Eucheuma*, with common species including *E. denticulatum* and *E. muricatum*, predominantly produces ι-carrageenan or a mixture of ι-carrageenan with a small proportion of κ-carrageenan, which is used for thickeners and gelling agents in dairy products and desserts [9]. The genus *Betaphycus* is the major source of β-carrageenan; this non-gelling carrageenan is applied in specific industrial sectors [10] (Figure 1).

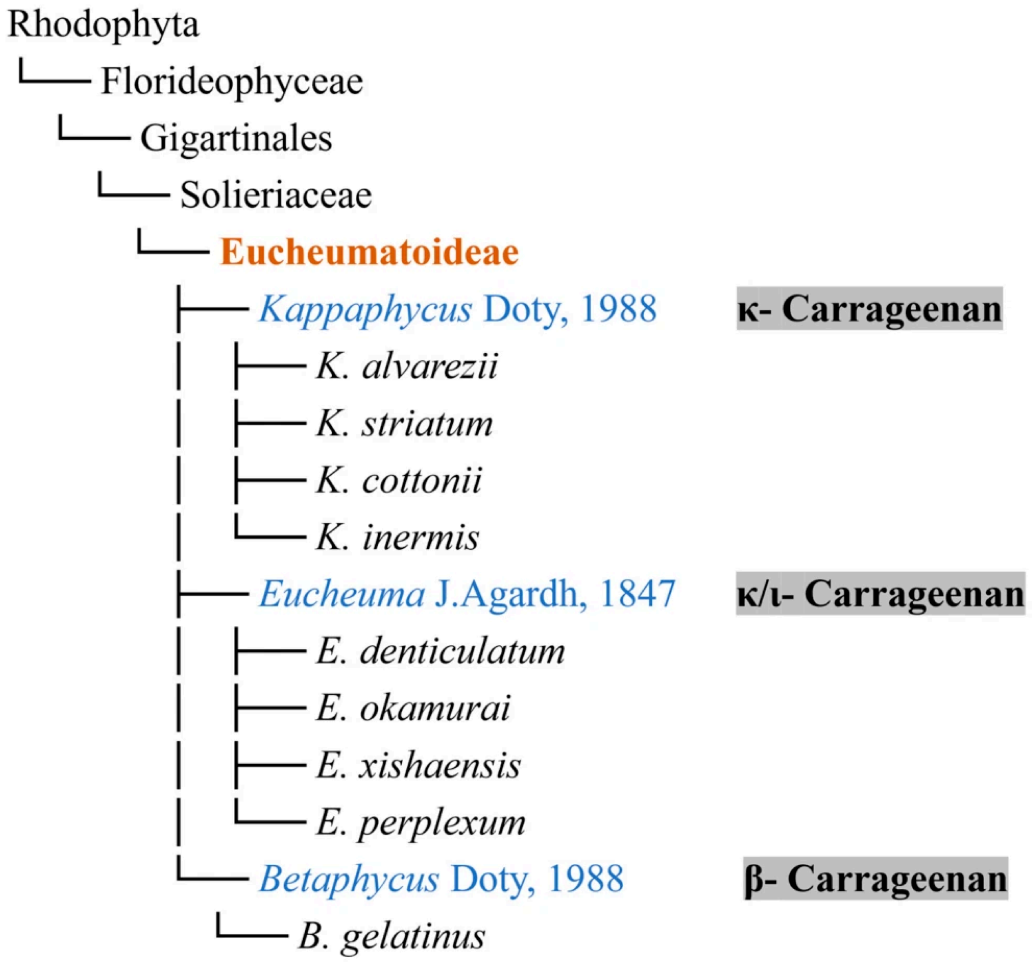


Figure 1. Phylogenetic placement and taxonomy of Eucheumatoid algae.

Traditional high-temperature alkaline extraction is the dominant industrial process for carrageenan production [11]. This well-established method drives the conversion of 6-sulfated galactose residues to 3,6-anhydrogalactose moieties via strong alkali treatment, significantly enhancing gel strength and product homogeneity [12]. Driven by environmental principles, ultrasound-assisted enzymatic extraction has emerged as a key research focus. It degrades algal cell wall components via hydrolytic enzymes (e.g., cellulase, protease) and facilitates intracellular carrageenan release through ultrasonic cavitation. With mild reaction conditions, this approach preserves polysaccharide structural integrity and native functional groups (e.g., sulfate groups) with lower environmental impact [13]. However, existing studies largely focus on process optimization for single species. Systematic cross-species comparisons of carrageenan quality responses to the two extraction methods across commercial Eucheumatoid algae remain limited.

Salinity fluctuation poses a major threat to the supply stability of the carrageenan industry in rainfall-prone tropical coastal production zones [14]. As a core environmental regulator of osmotic homeostasis and polysaccharide biosynthesis in red algae, salinity induces adaptive alterations in the biosynthetic pathways, degree of sulfate substitution, and molecular conformation of carrageenan, which serves as a key structural component of the algal cell wall and a critical osmolyte, thereby directly altering core physicochemical properties of the final product, including gel strength, viscosity, and textural parameters [15]. Current studies of Eucheumatoid have predominantly focused on salinity effects on the growth and physiological–biochemical traits. Systematic characterizations of carrageenan quality shifts across multiple commercial species under low-salinity gradients remain scarce. Furthermore, the combined regulatory mechanism underlying the interaction between salinity stress and extraction methods on carrageenan type composition and functional properties remains incompletely elucidated.

Heavy rainfall and fluvial freshwater runoff brought about by the monsoon season in Hainan cause sharp salinity declines in coastal waters, constituting one of the major environmental stresses constraining tropical seaweed cultivation [16]. Low-salinity stress disrupts the osmotic balance of algal thalli, alters cell turgor pressure, and further impairs cellular metabolism, possibly even leading to algal death [17]. Aslan et al. [18] investigated the seasonal variations in growth rate and carrageenan yield of *K. alvarezii* across different seasons in Indonesia, and confirmed that salinity fluctuation is one of the key factors driving seasonal yield differences. Kumar et al. [19] employed RNA-sequencing technology to explore the biochemical responses and gene expression profiles of *K. alvarezii* under low- and high-salinity conditions. Significant differences were observed in the accumulation of different osmoregulatory substances and photosynthetic pigments, while the activities of SOD, APX and GPX were markedly elevated under salinity stress.

Located in the tropical–subtropical area of China, Hainan boasts superior natural conditions for the cultivation of carrageenan-producing macroalgae. In this study, five major commercially available carrageenan-producing Eucheumatoid algae, Hainan green *Kappaphycus alvarezii* (Hn-G), Hainan red *Kappaphycus alvarezii* (Hn-R), Malaysia red *Kappaphycus alvarezii* (Ma-R), Malaysia *Kappaphycus striatum* (Ma-Ks) and Hainan *Eucheuma denticulatum* (Hn-E), were selected as experimental materials. The study aimed to comprehensively compare differences in the economic trait of carrageenan extracted from various germplasms via different extraction methods. In addition, we characterized the changes in carrageenan gel properties of different species under varying salinity stresses and evaluated the impacts of salinity fluctuation on carrageenan quality and cultivation benefits. Distinct from prior studies focusing largely on single species or single stress indicators, the novelty of this work lies in the simultaneous evaluation of multiple commercial Eucheumatoid germplasms, extraction approaches, and salinity-dependent carrageenan gel traits, which delivers inte-

grated germplasm-performance evidence. This work will help to screen macroalgae suited for carrageenan extraction under salinity-fluctuating environments, providing a scientific foundation for the development of the carrageenan industry.

2. Results

2.1. Effects of Alkaline Extraction and Ultrasound-Assisted Enzymatic Extraction on Carrageenan Yield, Physicochemical Properties and Textural Parameters of Five Eucheumatoid Algae

The extraction method used directly affects the yield and physicochemical properties of carrageenan. We extracted carrageenan from the five species using the two most widely adopted methods at present, namely alkaline extraction (AE) and ultrasound-assisted enzymatic extraction (UAEE). The result using Two-way ANOVA (Figure 2a, Tables S1 and S2) showed that the gel yield was significantly affected by species, extraction method, and their interaction ($p < 0.001$ for all terms). The AE method consistently delivered markedly higher gel yields than the UAEE method for Hn-G and Hn-R, exhibiting average enzymatic yields approximating 32–34%, whereas the AE yields remained below 18%. Substantial shifts in yield performance were observed for Ma-Ks, Ma-R and Hn-E. The UAEE method achieved drastically elevated gel yields for these three species, with median yields exceeding 34%, 42% and 45%, respectively. By contrast, the AE method produced much lower median yields (~17%, 16% and 27%, sequentially). Above all, Ma-R and Hn-E displayed wider yield fluctuations under AE treatment, while Hn-G, Hn-R and Ma-Ks presented narrow yield variance during UAEE.

Gel strength, sulfate content and viscosity were then measured to characterize the physicochemical properties of carrageenan obtained from the five Eucheumatoid species, and the carrageenan quality among them was compared. Two-way ANOVA (Tables S3 and S4) showed that gel strength was significantly affected by species, extraction method, and their interaction ($p < 0.001$ for all terms). The overall ranking of gel strength across both extraction methods followed the order Hn-E > Ma-R > Ma-Ks > Hn-R > Hn-G (Figure 2b). For Hn-G, Hn-R and Ma-Ks, gel strength when extracted using the AE method was significantly higher (195–202 g·cm^{-2}) than with UAEE (185–195 g·cm^{-2}). No statistically significant differences in gel strength were detected for Ma-R and Hn-E, with their median values ranging within 202–204 g·cm^{-2} and above 210 g·cm^{-2}, respectively.

The sulfate contents of carrageenan obtained via UAEE were significantly higher than those from AE across all tested cultivars (Figure 2c). Two-way ANOVA (Tables S5 and S6) showed that sulfate content of carrageenan was significantly affected by species, extraction method, and their interaction ($p < 0.001$ for all terms). The sulfate contents with the UAEE method ranged from approximately 23% to 32%, whereas AE markedly reduced the sulfate proportion (18–22%). The sulfate content of both extraction methods followed the descending order Hn-E > Ma-R > Ma-Ks > Hn-R > Hn-G, which was the same as the order of gel strength. Hn-E by ultrasound-assisted enzymatic extraction possessed the maximum sulfate content among all groups.

In terms of the viscosity, the AE method afforded better carrageenan than the UAEE method among the algae, except Ma-R (Figure 2d). Two-way ANOVA (Tables S7 and S8) showed that viscosity was significantly affected by species, extraction method, and their interaction ($p < 0.001$ for all terms). The differences were most significant for Hn-G and Hn-R, with the median viscosity of AE-samples ranging from 230 to 235 mPa·s, whereas UAEE counterparts had results below 190 mPa·s. Comparable viscosity was observed between the two approaches for Ma-Ks and Hn-E, with Hn-E exhibiting the highest overall viscosity (>250 mPa·s). In contrast, Ma-R displayed an opposite trend, in which UAEE yielded carrageenan with significantly higher viscosity than the AE method.

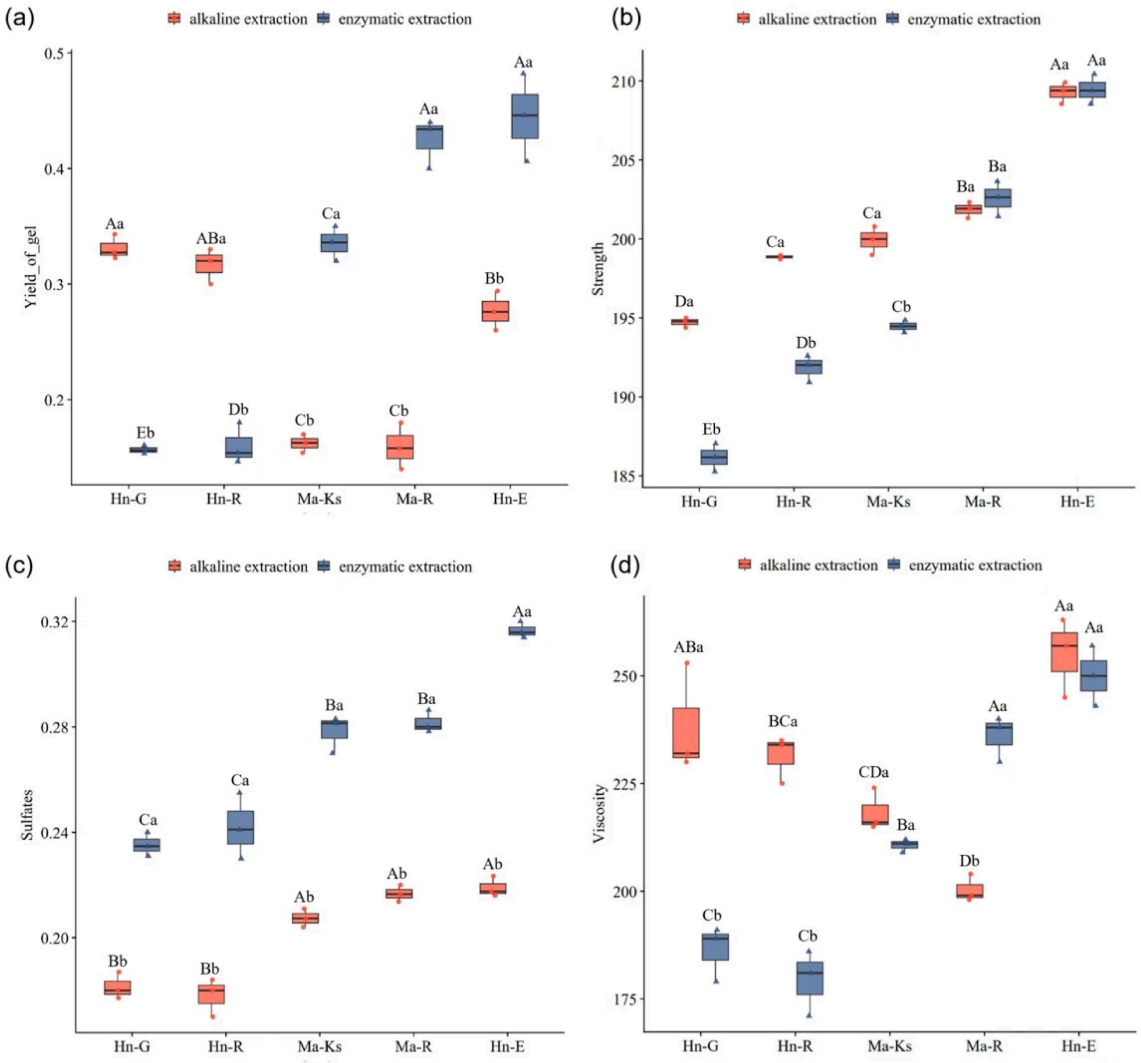


Figure 2. Comparison of gel yield and physicochemical properties obtained by alkaline extraction and ultrasound-assisted enzymatic extraction across five Eucheumatoid algae. (**a**) Yield of gel; (**b**) gel strength; (**c**) sulfate content; (**d**) viscosity. Uppercase letters denote significant differences among algal species under the same extraction method; lowercase letters denote significant differences between extraction methods for the same algal species ($p < 0.05$). Box plots show median value, interquartile range and raw replicate data of each index. Error bars represent the standard deviation of biological replicates ($n = 3$).

To investigate the differences in the physical and mechanical properties of carrageenan extracted using two extraction methods and from different algal species, five textural parameters (TPs) of carrageenan, hardness (Figure 3a), chewiness (Figure 3b), cohesiveness (Figure 3c), springiness (Figure 3d) and resilience (Figure 3e), were analyzed. The extraction method altered the textural parameters of carrageenan gels across the tested species. The textural parameters were significantly affected ($p < 0.001$ for all terms) by species, extraction method, and their interaction (Tables S9–S18). The parameters of carrageenan extracted by AE from the five algal species were all significantly higher than those obtained via UAEE, with the exception of Hn-E, for which the TPs showed nearly no significant difference between the two extraction methods. In addition, the overall ranking of TPs by UAEE methods followed the order Hn-E > Ma-R > Ma-Ks > Hn-R > Hn-G, with the significant differences between species ($p < 0.05$). The cohesiveness of Ma-R, and the springiness and resilience of Hn-R extracted by the AE method did not conform to the above trend.

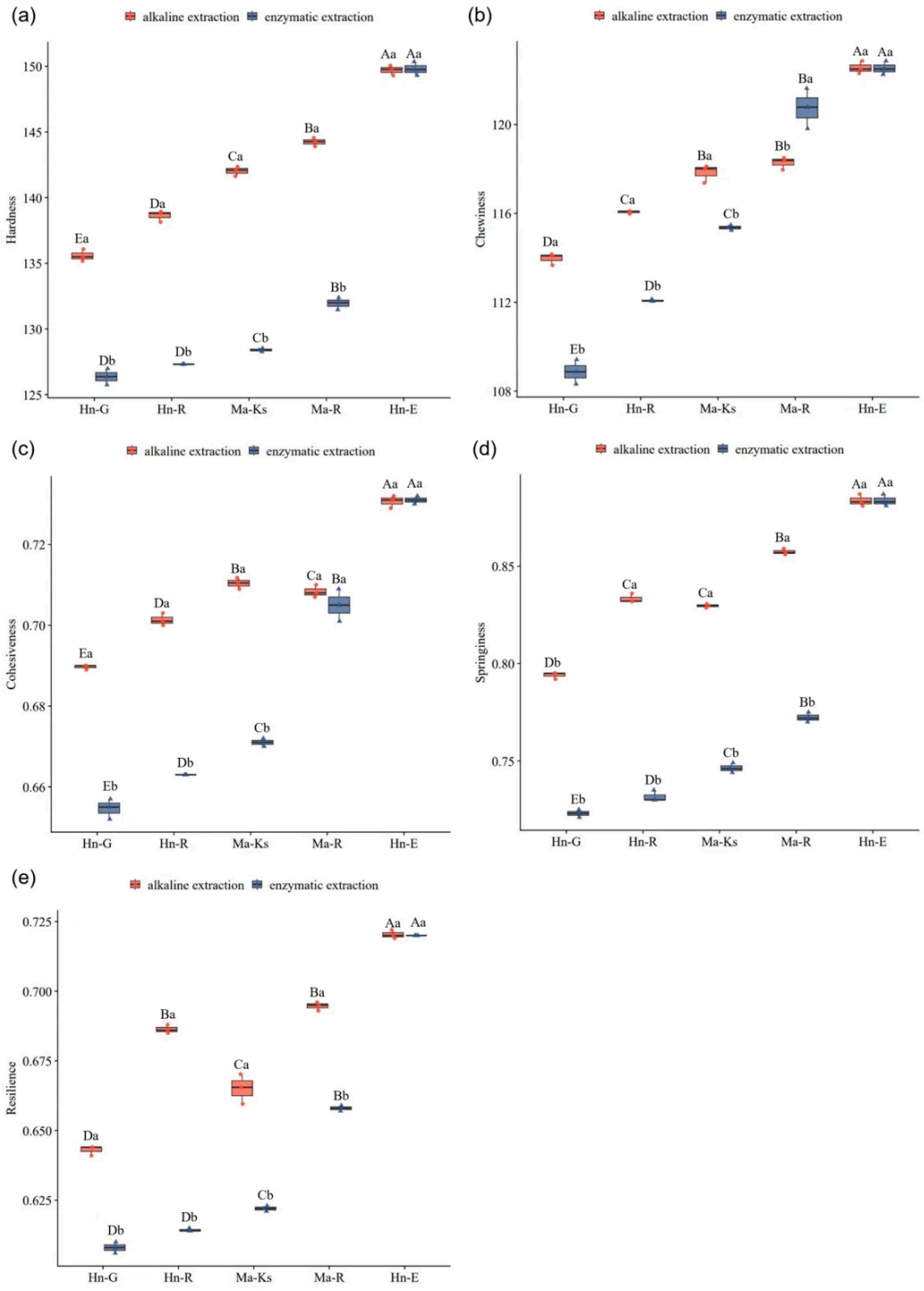


Figure 3. Textural parameters of carrageenan from five Eucheumatoid species prepared via alkaline extraction and ultrasound-assisted enzymatic extraction. (**a**) Hardness; (**b**) chewiness; (**c**) cohesiveness; (**d**) springiness; (**e**) resilience. Uppercase letters denote significant differences among algal species under the same extraction method; lowercase letters denote significant differences between extraction methods for the same algal species ($p < 0.05$). Box plots show median value, interquartile range and raw replicate data of each index. Error bars represent standard deviation of biological replicates ($n = 3$).

2.2. Fourier-Transform Infrared Spectroscopy Identification of Carrageenan Types Under Two Extraction Protocols

What are the carrageenan types of the five species? Are there any differences in carrageenan types obtained via the two extraction methods? Fourier-transform infrared (FTIR) spectra were used to identify characteristic functional groups of carrageenans. The diagnostic absorption bands were assigned as follows (Table 1): the broad band near 3350 cm^{-1} corresponds to O–H stretching of hydroxyl groups; the peak around 2920 cm^{-1} is attributed to C–H stretching of aliphatic sugars; the absorption at approximately 1220–1260 cm^{-1} arises from S=O asymmetric stretching of sulfate esters; the band near 990–1160 cm^{-1} is assigned to C–O stretching of pyranose rings; the peak at ~930 cm^{-1} represents the C–O–C vibration of 3,6-anhydrogalactose; and the absorption around 845 cm^{-1} is characteristic of axial C–O–S stretching of galactose sulfate.

Table 1. Assignment of FTIR characteristic peaks of carrageenan.

Wavenumber (cm^{-1})	Functional Group Assignment	Core Significance
3300–3400	O-H stretching vibration of polysaccharide hydroxyl groups	Reflects hydrogen bonding, water content and exposure degree of free hydroxyl groups
2910–2930	C–H stretching vibration of aliphatic sugar rings	Indicates the structural integrity of polysaccharide carbon skeleton
1640–1650	Bending vibration of bound water/C=O vibration	Represents bound water content and molecular polarity of samples
1210–1260	O=S=O asymmetric stretching vibration of sulfate ester groups	Characteristic peak for total sulfate content; higher peak intensity corresponds to higher sulfate substitution degree
1150–1160	C-O stretching vibration of pyranose rings	Evaluates the structural integrity of the polysaccharide main chain
990–1100	C-O stretching vibration of pyranose rings	Evaluates the structural integrity of the polysaccharide main chain
920–930	C–O–C vibration of 3,6-anhydrogalactose	Marker peak of κ-carrageenan; higher peak intensity means stronger gel-forming capacity
845–850	C–O–S stretching of galactose sulfate	Diagnostic peak of κ-type carrageenan from *Eucheuma denticulatum*
805	2-sulfated galactose vibration	Diagnostic peak of ι-type carrageenan from *Kappaphycus* species

The absorption bands at 3350, 2920, 1240, and 1060 cm^{-1} confirm the presence of carrageenan in the samples, whereas peaks at 930, 845, and 805 cm^{-1} are used for carrageenan typing. The detection of peaks at 930 and 845 cm^{-1} with no obvious signal at 805 cm^{-1} indicated κ-carrageenan. The simultaneous occurrence of peaks at 930, 845, and 805 cm^{-1} was diagnostic for ι-carrageenan. A very weak or absent 930 cm^{-1} peak, together with

the absence of peaks at 845 and 805 cm^{-1} and a broad absorption band at 820–830 cm^{-1}, suggested λ-carrageenan.

When using the alkaline extraction method (red lines, Figure 4), distinct absorption bands were observed at wavenumbers of approximately 3326.5–3368.7 cm^{-1}, 2917.9–2927 cm^{-1}, 1641.8–1647 cm^{-1}, 1215.6–1258 cm^{-1}, 1149.3–1156.8 cm^{-1}, 994.2–1075 cm^{-1}, 923.8–929 cm^{-1} and 845–848.1 cm^{-1}. This result indicated that all five species contained κ-type carrageenan, while λ-type carrageenan was absent in all samples. Additionally, trace amounts of ι-type carrageenan existed in carrageenan extracted from Hn-R.

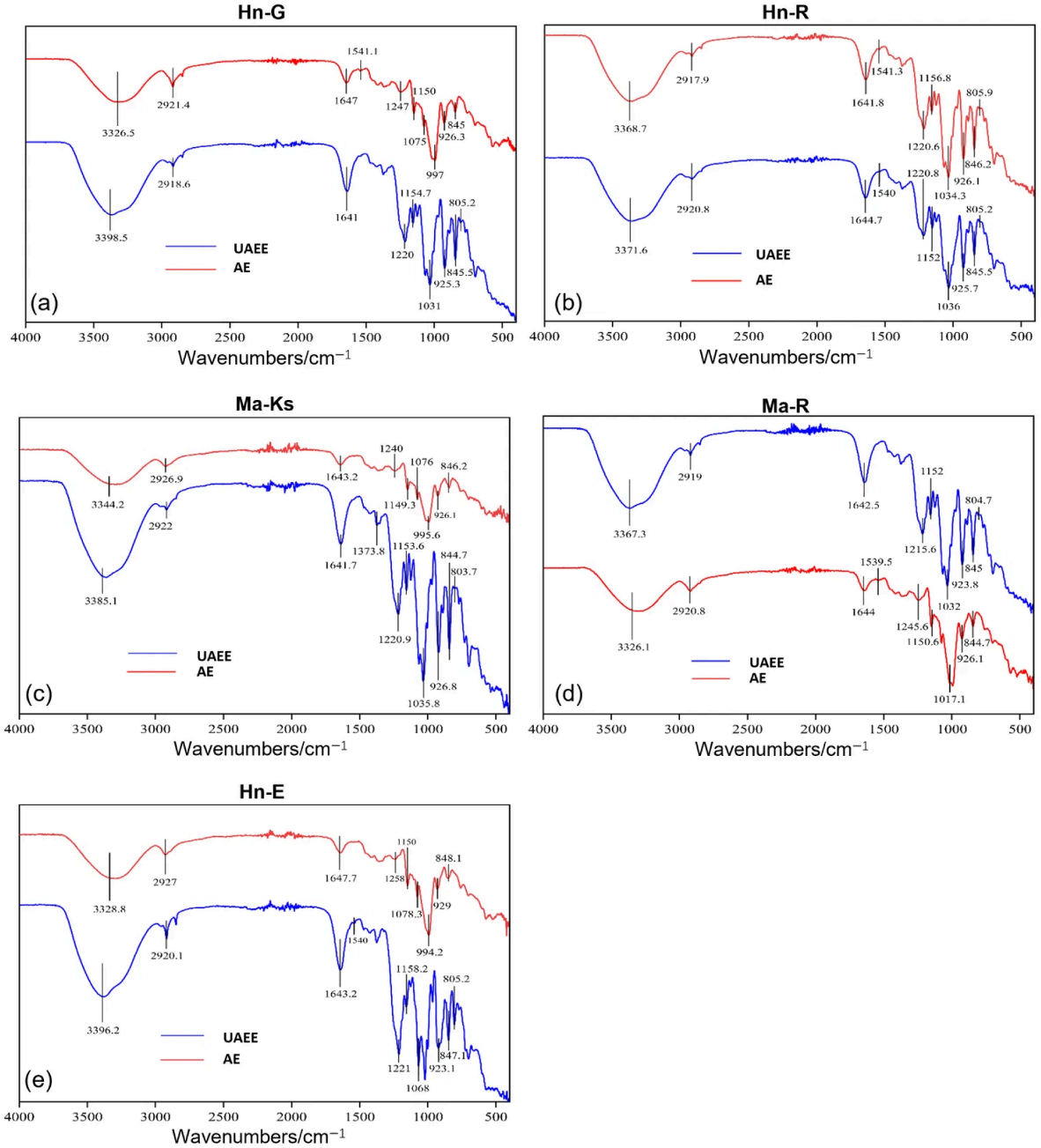


Figure 4. Fourier-transform infrared spectroscopy of carrageenan from Hn-G (**a**), Hn-R (**b**), Ma-Ks (**c**), Ma-R (**d**) and Hn-E (**e**) obtained by UAEE and AE methods.

When using ultrasound-assisted enzymatic extraction (blue lines, Figure 4), significant absorption bands were observed at approximately 3326.1–3398.5 cm^{-1}, 2918.6–2922 cm^{-1},

1641–1644.7 cm^{-1}, 1220–1245.6 cm^{-1}, 1150.6–1158.2 cm^{-1}, 1017.1–1036 cm^{-1}, 923.1–926.8 cm^{-1} and 844.7–847.1 cm^{-1} in the FTIR spectra of the five samples. This result suggested that κ- and ι-type carrageenan were detected in all five species using the ultrasound-assisted enzymatic extraction method, and Hn-E exhibited the highest peak value. No λ-type carrageenan was found in any of the five algae. Protein signals were detected in samples of Hn-R and Hn-E.

2.3. Short-Term Low-Salinity Stress Effects on Carrageenan Yield, Physicochemical Properties and Textural Parameters

Does abnormal salinity affect the gel content and physicochemical properties of five algae? What is the variation trend of textural performances with declining salinity? To investigate the short-term effects of low-salinity stress on carrageenan extracted from the five Eucheumatoids, three salinity gradients—18‰, 24‰ and 32‰—were set to measure the yield of gel, physicochemical properties and textural parameters for twelve days. The algal thalli cultured under low-salinity conditions died on the 12th day, preventing carrageenan extraction and measurements. Therefore, this point was selected for analysis (Table 2).

Table 2. Survival status of five algal species under different low-salinity conditions.

Salinity		Day 0	Day 3	Day 6	Day 9	Day 12
18‰	Hn-G	L	L	W	W	W
	Hn-R	L	W	W	Y	Y
	Ma-Ks	L	W	W	Y	Y
	Ma-R	L	Y	Y	Y	D
	Hn-E	L	W	W	W	Y
24‰	Hn-G	L	L	L	W	W
	Hn-R	L	L	L	W	W
	Ma-Ks	L	L	W	W	Y
	Ma-R	L	W	W	W	Y
	Hn-E	L	L	W	W	Y
32‰	Hn-G	L	L	L	L	L
	Hn-R	L	L	L	L	L
	Ma-Ks	L	L	L	L	L
	Ma-R	L	L	L	L	L
	Hn-E	L	L	L	L	L

Note: L indicates healthy condition, W indicates slight decay, Y indicates severe decay, and D indicates death.

The carrageenan yield was significantly affected ($p < 0.001$ for all terms) by species, salinity, and their interaction (Tables S19 and S20). For Hn-G, Hn-R, Ma-Ks and Hn-E, carrageenan content increased with rising salinity, and 18‰ significantly suppressed extracted gel content (Figure 5a). Ma-R showed a unique trend, whereby its maximum carrageenan yield occurred at 18‰, whereas elevated salinity markedly reduced gel production. Inter-species comparison at identical salinity showed that Ma-R yielded the highest gel under 18‰, and at 24‰ and 32‰, Hn-E possessed superior carrageenan content. Hn-R had the lowest yield under three salinity conditions. Above all, low salinity at 18‰ suppressed carrageenan accumulation in most algal species yet elevated the gel yield of the Ma-R, indicating that Ma-R exhibited superior gel-production performance under hypo-saline stress.

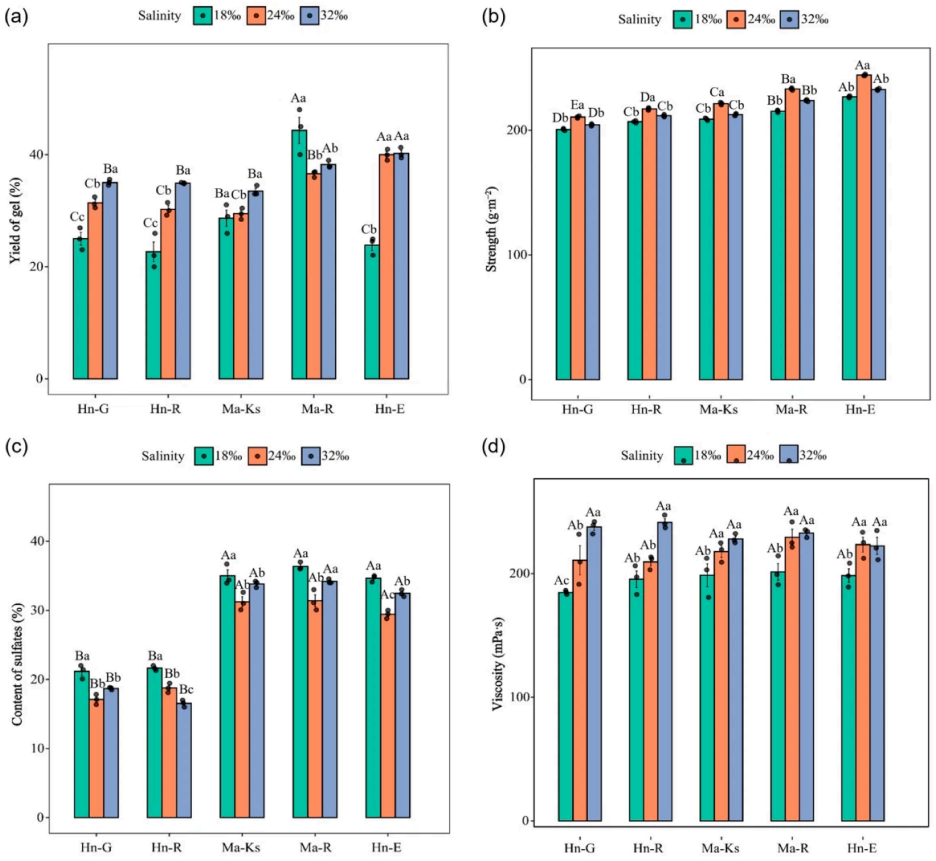


Figure 5. Carrageenan yield and physicochemical properties extracted from five Eucheumatoids cultivated at three salinity levels: 18‰, 24‰, and 32‰. (**a**) Yield of gel; (**b**) gel strength; (**c**) sulfate content; (**d**) viscosity. Uppercase letters denote significant differences among algal species at the same salinity; lowercase letters denote significant differences among salinity treatments for the same algal species ($p < 0.05$). Box plots show median value, interquartile range and raw replicate data of each index. Error bars represent standard deviation of biological replicates ($n = 3$).

Low salinity significantly modulates the physicochemical properties of carrageenan from five Eucheumatoids. Two-way ANOVA (Tables S21–S26) showed that gel strength and sulfate content were significantly affected by species, salinity, and their interaction ($p < 0.001$ for all terms). By contrast, viscosity was only significantly affected by salinity, with no significant effects of species or the species × salinity interaction. The gel strength (Figure 5b) of all species peaked at 24‰ and mildly dropped at 32‰. Hn-E had the highest gel strength across all salinities, while Hn-G showed the lowest. Low salinity (18‰) significantly weakened the gel strength for all genotypes. The sulfate contents (Figure 5c) of carrageenan in Hn-G and Hn-R were distinctly lower than Ma-Ks, Ma-R and Hn-E. Low-salinity 18‰ promoted sulfate synthesis in all tested algae. The viscosity (Figure 5d) continuously increased along with elevated salinity, and the order was 32‰ > 24‰ > 18‰. Hn-G and Hn-R achieved higher viscosity at 32‰, and Mar-R achieved higher viscosity at 18‰ and 24‰.

It was observed that increasing salinity improved gel strength (peaking at 24‰) and viscosity (peaking at 32‰), but suppressed sulfate accumulation. Ma-R and Hn-E exhibited superior overall carrageenan quality relative to the other three algal thalli.

The textural properties of carrageenan from five Eucheumatoids also appeared to be significantly affected by low-salinity stress, with consistent trends across all species (Figure 6). Across all genotypes, hardness (Figure 6a), chewiness (Figure 6b), cohesiveness (Figure 6c), springiness (Figure 6d), and resilience (Figure 6e) were lowest at 18‰, peaked

at 24‰, and slightly decreased at 32‰. The differences among salinity treatments were significant or highly significant for each alga. When compared across species under the same salinity, Hn-E exhibited the highest values in all textural properties, followed by Ma-R, while Hn-G generally showed the weakest gel textural properties. Notably, the magnitude of salinity-induced changes was most pronounced in Ma-R and Hn-E, indicating strong genotype-dependent responses to salinity fluctuation. Detailed two-way ANOVA results for individual textural parameters were shown in Tables S27–S36.

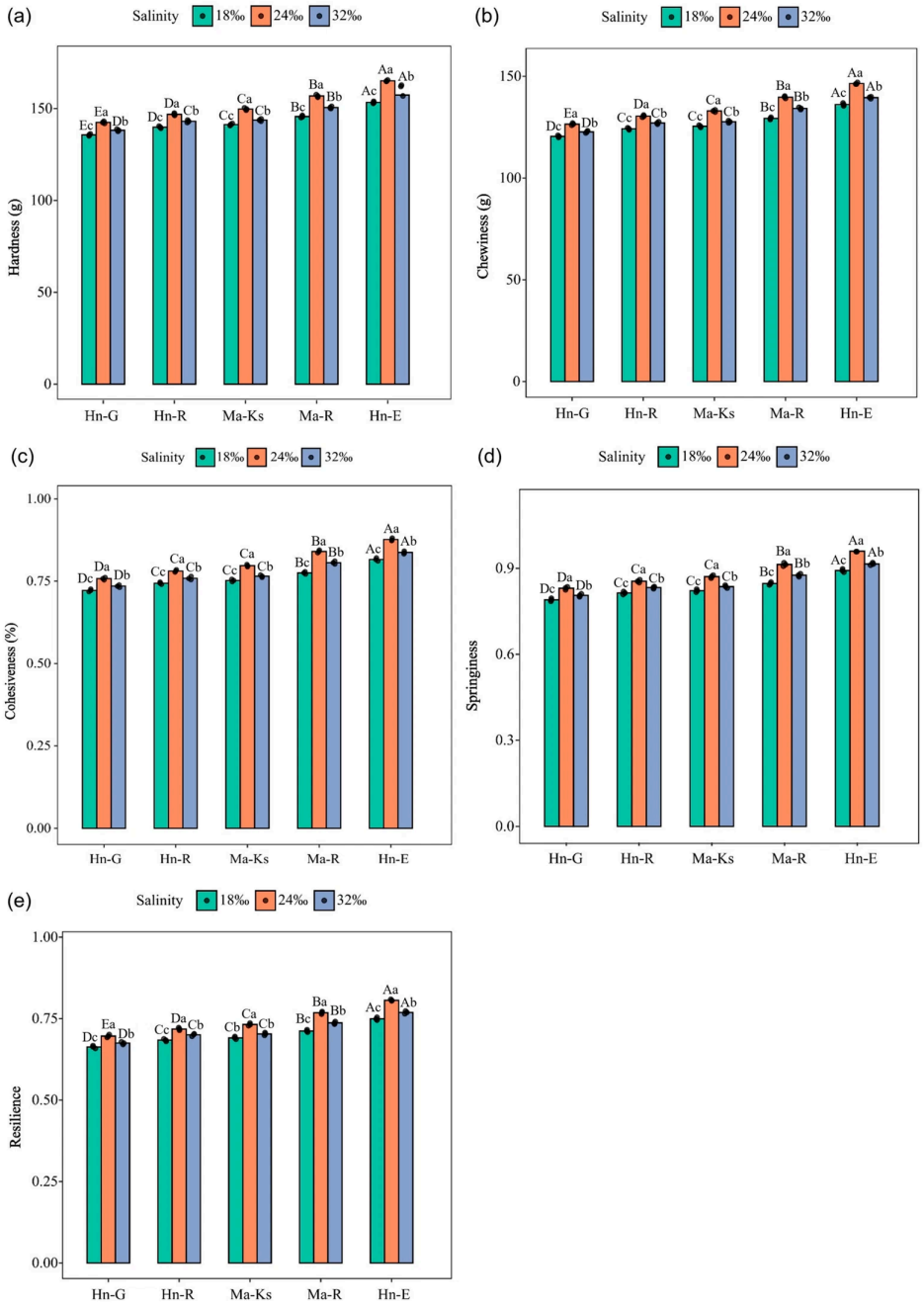


Figure 6. Gel textural parameters of carrageenan from five Eucheumatoid algae cultivated at three salinities levels: 18‰, 24‰, and 32‰. (**a**) Hardness; (**b**) chewiness; (**c**) cohesiveness; (**d**) springiness; (**e**) resilience. Uppercase letters denote significant differences among algal species at the same salinity; lowercase letters denote significant differences among salinity treatments for the same algal species ($p < 0.05$). Box plots show median value, interquartile range and raw replicate data of each index. Error bars represent standard deviation of biological replicates ($n = 3$).

3. Discussion

This study systematically compared the effects of AE and UAEE methods on the yield and quality of carrageenan from five Eucheumatoids. Combined with low-salinity stress treatments, this study revealed interspecific differences in the physicochemical properties of carrageenan from distinct thalli and their responses to low salinity.

3.1. Effects of Extraction Methods on Carrageenan Yield and Quality

Extraction methods exerted significant influences on the yield and properties of carrageenan (Figure 2, Tables S1–S8). UAEE method yielded remarkably higher carrageenan recovery than AE for Ma-R, Ma-Ks, and Hn-E (Figure 2a). This observation was consistent with the findings [20] of that cellulase and hemicellulase synergistically disrupted cell wall architectures, fully releasing intracellular carrageenan and improving extraction efficiency under relatively mild reaction conditions, with higher strength and sulfate contents. The yield, strength and sulfate contents were all higher by UAEE method in Ma-R, Ma-Ks, and Hn-E (Figure 2b–d). From an operational perspective, UAEE generally requires a lower alkali concentration and a shorter processing time, which can reduce energy consumption and mitigate excessive alkaline degradation of polysaccharides with the high total yield. Nevertheless, this method has clear limitations: the co-extraction of impurities such as proteins frequently occurs during enzymatic treatment, which reduces the purity of the final products. In this study, FTIR spectra detected characteristic protein signals in UAEE extracts of Hn-R and Hn-E (Figure 4), further verifying that enzymatic extraction might introduce protein contaminants. In addition, if cellulose, hemicellulose and pectin are scarce in the algal cell wall matrix, the cell wall cannot be disrupted by enzymes, and the extraction efficiency of the UAEE method will be inferior to that of the alkaline method.

By contrast, AE exhibited prominent advantages for *K. alvarezii* from Hainan (Hn-G and Hn-R) (Figure 2a). This is because the cell walls of *Kappaphycus alvarezii* in Hainan contain low levels of cellulose, hemicellulose and pectin. Cell cross-linking is maintained by phenolic and protein networks, leading to poor cell disruption efficiency via UAEE method. Hilliou [21] reported that κ-carrageenan recovered via conventional alkaline extraction generally possessed elevated amounts of 3,6-anhydrogalactose (3,6-AG) and reduced sulfate groups. Additionally, Ferdiansyah [22] adopted alkaline extraction for carrageenan from *Eucheuma* species, and achieved 45.08% and 80.9% increments in 3,6-AG content and gel strength, respectively. These results aligned well with our findings, where AE demonstrated superior gel strength (Figure 2b) and viscosity (Figure 2d), and less sulfate (Figure 2c). Beyond gel performance, AE is a mature, scalable industrial route with well-established operating protocols and low reagent cost. However, alkaline extraction also has inherent drawbacks: harsh alkaline conditions may cause partial polysaccharide degradation if temperature or duration is poorly controlled, and the high pH environment generates wastewater, requiring neutralization and treatment and therefore increasing downstream environmental and processing burdens.

In the same species, except for Hn-E, carrageenan extracted by AE displayed significantly superior performance in hardness, chewiness, cohesiveness, springiness, and resilience compared with carrageen extracted using the UAEE method (Figure 3, Tables S9–S18). A previous study [23] demonstrated that gel strength was positively correlated with 3,6-AG content and negatively correlated with sulfate content. But in this study, such an observed correlation does not establish a direct causal relationship. Gel strength may be jointly modulated by multiple interconnected factors, including carrageenan chemotype, molecular weight distribution, ionic composition of the gel system, and standardized gel-preparation conditions. Sulfation status represents only one contributing variable. For industrial production, process selection should be tailored to raw algal materials and target

product attributes. UAEE is suitable for scenarios prioritizing high extraction yield, mild reaction conditions and energy saving, though subsequent purification steps may be necessary to remove protein impurities. AE is optimal when high gel strength and desirable textural quality are the primary targets, particularly for germplasms including Ma-R, Ma-Ks and Hn-E, while wastewater treatment and precise process control must be considered for large-scale application.

3.2. Interspecific Variations in Carrageenan Chemotypes

FTIR spectroscopic analysis (Figure 4 and Table 1) indicated that carrageenan isolated from all five Eucheumatoids was dominated by κ-type fractions accompanied by partial ι-carrageenan, whereas λ-carrageenan was not detected in any sample and extraction method. Among all species, Hn-E contained the highest proportion of ι-carrageenan by the UAEE method. This outcome complied with the established taxonomic traits of Eucheumatoid algae: the genus *Kappaphycus* (including *K. alvarezii* and *K. striatum*) primarily biosynthesizes κ-carrageenan, while the genus *Eucheuma* (represented by *E. denticulatum*) accumulated ι-carrageenan or κ/ι hybrid carrageenan. But in the AE method, signal of ι-carrageenan was not detected, implying that the duration of our alkaline treatment may have been excessive. Alkaline extraction is also referred to as the κ-carrageenan-favoring extraction method, since prolonged alkaline treatment eliminates C2-sulfate substituents on ι-carrageenan and progressively transforms ι-units into κ-like structural motifs, and thereby may alter or mask the diagnostic FTIR signals of ι-carrageenan in Hn-E. In this study, minor discrepancies in impurity signals were observed between AE and UAEE extracts. Protein absorbance peaks were identified in AE extracts of Hn-G, Hn-R and Ma-R, whereas proteins were predominantly detected in UAEE extracts of Hn-R and Hn-E. This might be attributed to the co-extraction of tightly bound protein-polysaccharide conjugates during alkaline extraction. In contrast, ultrasonic treatment for enzymatic extraction might denature partial proteins and trigger the formation of novel protein–polysaccharide complexes with polysaccharides. It should be noted that FTIR provides qualitative information on major functional groups of carrageenan. However, this technique has inherent limitations for discriminating closely related carrageenan structures or mixed carrageenan samples, because subtle differences in sulfation patterns and the relative proportions of κ-, ι-, and λ-carrageenan may produce highly similar spectral profiles. Therefore, FTIR in the present study serves as a preliminary qualitative characterization, and complementary methods such as NMR are required to resolve mixed or structurally analogous carrageenan. Nevertheless, FTIR has limited resolving power to differentiate structurally similar carrageenan or their mixtures, as minor variations in substitution patterns can yield largely overlapping spectra. Accordingly, here, FTIR results are interpreted only as qualitative evidence rather than definitive identification of individual carrageenan fractions.

3.3. Effects of Low-Salinity Stress on the Yield and Quality of Carrageenan

Low-salinity stress acts as a critical environmental factor regulating carrageenan biosynthesis and quality, as reported previously [24]. In this study, carrageenan contents of Hn-G, Hn-R, Ma-Ks and Hn-E were markedly suppressed under 18‰ low-salinity stress. In contrast, Ma-R showed a distinct response pattern, with its carrageenan yield peaking at 18‰ and gradually decreasing as salinity increased (Figure 5a, Tables S19 and S20). We hypothesized that this pattern might arise because Ma-R improved cell wall polysaccharide synthesis under hypotonic conditions, possibly to maintain intracellular osmotic balance and mitigate cellular water loss under low ambient salinity. A previous study [25] documented the differential expression of numerous genes involved in redox homeostasis in *K. alvarezii* under low salinity exposure, alongside elevated SOD, APX and GPX activities. Thus, the

high carrageenan productivity of Ma-R under low salinity might be linked to a unique gene regulatory network and effective antioxidant defenses, though this remains to be verified. Collectively, Ma-R represented a promising germplasm candidate for carrageenan manufacturing of low-salinity tolerance.

Low-salinity stress also substantially remodeled the characteristics of carrageenan (Figure 5b–d, Tables S21–S26). For all tested algal species, gel strength reached the maximum at 24‰ and slightly decreased at 32‰. Sulfate content peaked at the lowest salinity level, showing an entirely opposite trend to gel strength, while viscosity rose continuously as salinity increased. Ha [26] validated the negative correlation between sulfate content and gel strength: excessive sulfate groups amplified electrostatic repulsion between polysaccharide chains, disrupted gel network formation, and consequently reduced gel strength. In this study, the five algae might elevate the sulfation degree of carrageenan to enhance water retention capacity and alleviate osmotic stress under hypotonic environments. Nevertheless, over-accumulated sulfate groups hindered intramolecular cyclization to form 3,6-AG, which ultimately deteriorated gel performance. Nevertheless, this covariation should not be simplified as a direct causal link. The final gel performance depends collectively on carrageenan chemotype, molecular weight, endogenous cation pools, and standardized gel-forming procedures. Viscosity is modulated interactively by sulfate groups, 3,6-AG and endogenous cations [6]. Cations screen sulfate repulsion to assist gel junction formation, and simultaneously promote interchain association that raises solution viscosity. This cation-mediated bridging effect creates an environment-dependent pattern of viscosity under changing salinity. This is consistent with the trends in our result: Hn-G and Hn-R had lower sulfate contents relative to the other three algal species and thus displayed higher viscosity. In addition, under elevated salinity, the accumulation of 3,6-anhydrogalactose and increased availability of free cations favor sustained interchain association, which drives the continuous increase in viscosity.

Textural properties (Figure 6, Tables S27–S36) revealed consistent salinity-dependent variations across all measured indices: hardness, chewiness, cohesiveness, springiness and resilience all attained maximum values at 24‰ and minimum values at 18‰. This uniform trend across all five thalli suggested that 24‰ was the best-performing salinity level among those tested for carrageenan quality in tropical Eucheumatoids. Siddiqui [27] reported that extreme low salinity (below 22‰) rapidly impaired Photosystem II function in *K. alvarezii*, whereas moderate low salinity (22–28‰) was tolerated over short-term cultivation periods. The 18‰ salinity treatment (approximately 18‰) adopted in this study approached the physiological tolerance limit of most species, leading to thalli and even death (Table 2), which restrained carrageenan synthesis and accumulation.

3.4. Comprehensive Interspecific Quality Evaluation and Cultivation Implications

Finally, we performed a comprehensive analysis of the physicochemical properties and textural parameters. The correlation heatmap of physicochemical and textural parameters (Figure 7a) revealed that physicochemical properties exhibited weak correlations ($r = -0.06$–0.50) with all other measured parameters, while all textural parameters showed an extremely strong positive correlation with each other ($r = 0.99$–1.00). The salinity-clustered heatmap (Figure 7b) indicated that only algae cultured at 32‰ salinity possessed high viscosity values (Column 1, Figure 7b). The gel yield of Ma-R and Hn-E was markedly higher than that of other species, with Ma-R at 18‰ salinity yielding the maximum gel content (Column 2). Regardless of salinity level, Hn-E exhibited the optimal performance across all five textural parameters (Columns 3–8).

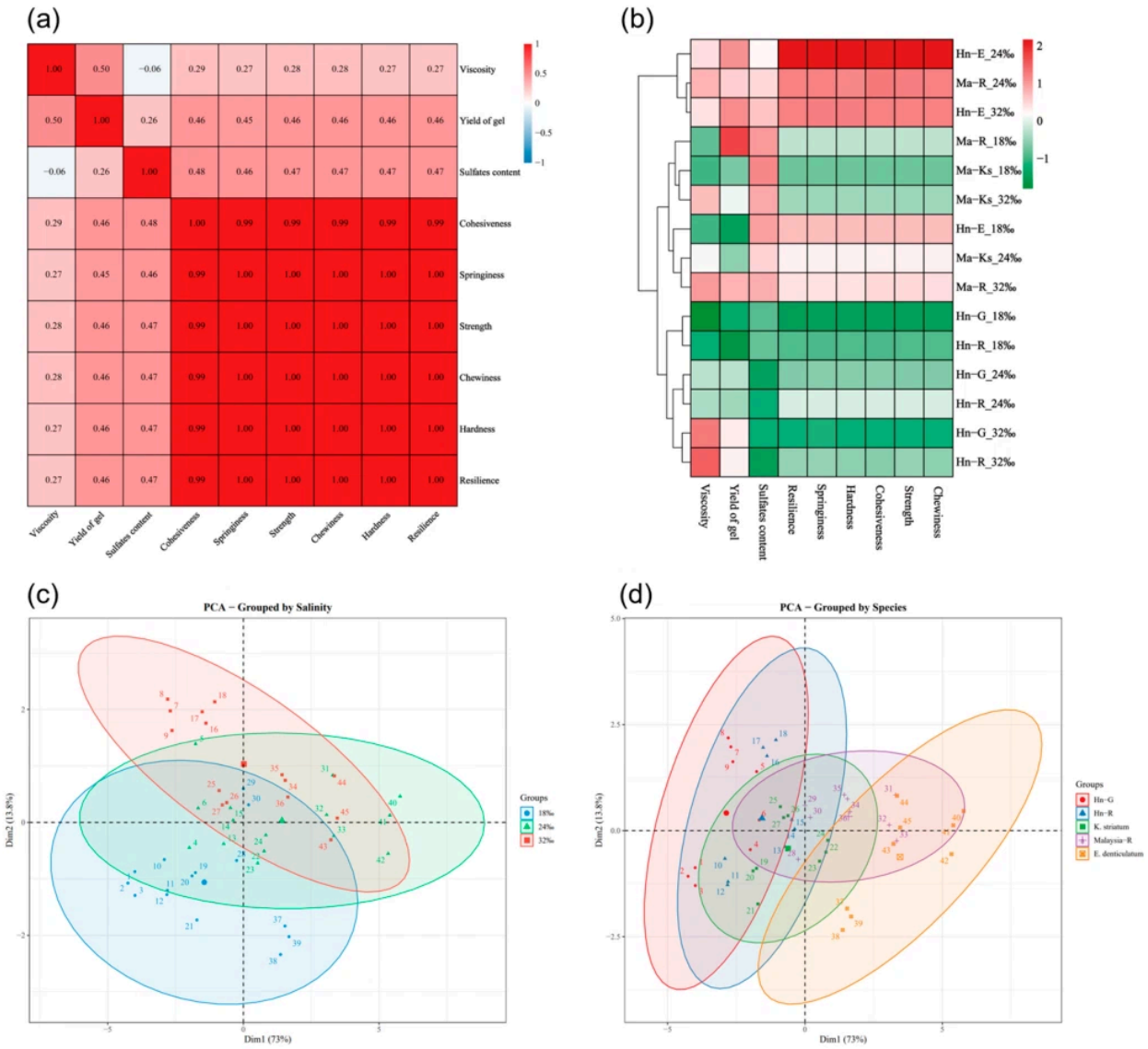


Figure 7. Multivariate analyses of physicochemical parameters of carrageenan across five Eucheumatoid species under three salinity treatments. (**a**) Pearson correlation matrix of carrageenan parameters. The color gradient represents the correlation coefficient (r), with red indicating positive correlation and blue indicating negative correlation. (**b**) Hierarchical clustering heatmap. Rows represent samples (species × salinity), and columns represent the parameters. The color scale reflects normalized values (red = high, blue = low). (**c**) Principal component analysis score plot grouped by salinity (18‰, 24‰, 32‰). Dim1 and Dim2 explain 73% and 13.8% of the total variance, respectively. Ellipses denote 95% confidence intervals for each salinity group. (**d**) PCA score plot grouped by algal species (Hn-G, Hn-R, Ma-Ks, Ma-R, Hn-E). Ellipses denote 95% confidence intervals for each species group.

Principal component analysis (PCA) grouped by salinity (Figure 7c) showed that samples grown at 18‰ clustered in the negative range of Dim1, samples at 32‰ clustered in the positive Dim1 range, and samples at 24‰ occupied an intermediate position between the two groups. This verified that salinity gradient acted as a key driver of divergence in carrageenan's physicochemical traits, with distinct characteristic separation among low-, medium-, and high-salinity treated samples. PCA grouped by algal species (Figure 7d) displayed no overlap between Hn-G and Hn-E, demonstrating prominent phenotypic differentiation between the two species.

Collectively, these analyses suggest that carrageenan yield and physicochemical traits co-vary synergistically with all textural properties (excluding viscosity), and all textural

parameters are highly interlinked. In addition, salinity above 24‰ is more conducive to the growth of most algae and the formation of favorable carrageenan. Both salinity and species significantly alter carrageenan yield, physicochemical characteristics, and textural parameters.

Integrating multiple evaluation indicators including carrageenan yield, gel strength, sulfate content, viscosity and textural attributes, the comprehensive quality ranking of the five Eucheumatoid algae was determined as Hn-E > Ma-R > Ma-Ks > Hn-R > Hn-G. Across all salinity treatments, Hn-E exhibited the highest gel strength, maximum sulfate content and optimal textural parameters, which was closely linked to the molecular architecture of its κ/ι hybrid carrageenan. The C2-sulfate moieties on ι-carrageenan conferred soft, elastic gel textures, which complement the rigid, brittle properties of κ-carrageenan and could expand the industrial application scope of Hn-E in manufacturing [6]. The carrageenan of Ma-R demonstrated the strongest low-salinity adaptability. At 18‰ low salinity, Ma-R achieved the highest carrageenan yield with relatively mild declines in gel strength and textural properties, demonstrating its cultivation advantages in low-salinity habitats such as estuarine regions and rainy seasons. Therefore, for Eucheumatoid aquaculture in tropical monsoon zones such as Hainan, farmers should fully anticipate abrupt salinity drops induced by freshwater runoff during rainy seasons. Low-salinity-tolerant germplasms should be prioritized for cultivation, or corresponding farming management strategies should be implemented to buffer salinity variations.

4. Materials and Methods

4.1. Materials and Cultivation

The experimental materials included Hainan green *Kappaphycus alvarezii* (Hn-G), Hainan red *Kappaphycus alvarezii* (Hn-R), Malaysia red *Kappaphycus alvarezii* (Ma-R), Malaysia *Kappaphycus striatum* (Ma-Ks) and Hainan *Eucheuma denticulatum* (Hn-E). All the above algal materials were cultured and preserved at Li'an Port, Lingshui County, Hainan Province (18°43′ N, 110°06′ E). The culture conditions were set as follows: water temperature of 28 °C, salinity of 35‰, and light intensity of 300 μmol·m^{-2}·s^{-1}. The Eucheumatoid algae used in this study are listed in Figure 8.

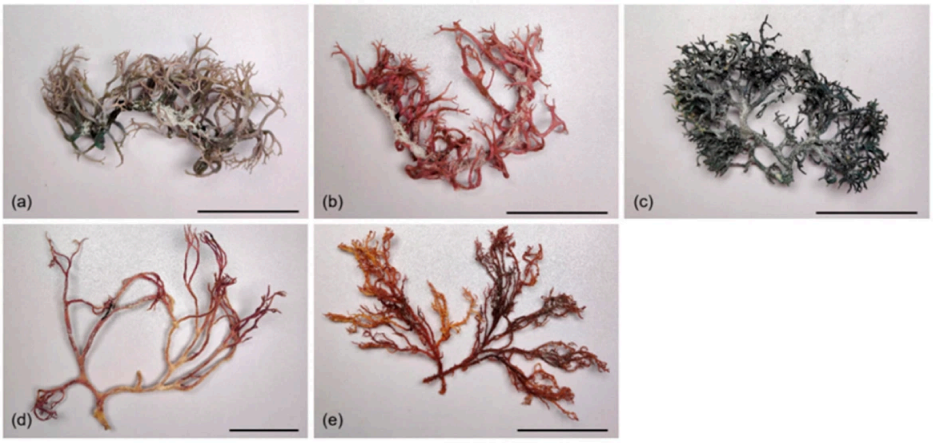


Figure 8. The dried Hn-G (**a**), Hn-R (**b**), Ma-Ks (**c**), Ma-R (**d**), and Hn-E (**e**).

The thalli for salinity experiments were acclimated for five days in natural seawater in culture tanks (60 cm × 30 cm× 39 cm; 30 L seawater) at 30 psu, water temperature 28 °C and light intensity 200 μmol·m^{-2}·s^{-1}. Following acclimation, algal thalli were randomly allocated to each salinity treatment and extraction method combination. There were 25 treatment combinations of algal species × salinity/extraction method. Three independent

culture tanks (n = 3 biological replicates) were set up for each combination, with each tank representing an independent experimental unit. Each tank contained five separate algal thalli, which were used for the determination of carrageenan yield, sulfate content, viscosity, and TPA and as reserved backup material. Algal samples were assigned to tanks and salinity treatments following a completely randomized design. The tanks were randomly positioned within the culture system to minimize positional bias. The initial fresh weight of each experimental thallus was 3.5 g, and the culture density was approximately 2 g·L^{-1}. Experimental salinities were prepared by mixing natural seawater collected from the coastal area near the Qionghai scientific research base (approximately 19.37° N, 110.67° E) with distilled water. The pH of the undiluted natural seawater before dilution ranged from 8.16 to 8.28 during the experimental period.

The salinity exposure experiment lasted for 12 days, and the experiment was terminated when thallus bleaching was first observed on Day 12 (Ma-R). Salinity was monitored daily with a refractometer (ATAGO, Tokyo, Japan), and distilled water or high-salinity seawater was supplemented to compensate for water evaporation and retain target salinity. Additionally, 20% of the culture medium was renewed every two days to supplement nutrients and prevent abrupt environmental fluctuations caused by complete water exchange, which may alter algal physiological and biochemical parameters. Continuous aeration was applied in all tanks throughout the whole cultivation period.

For carrageenan extraction, algal individuals with good growth status, intact thalli and no obvious diseases were selected. After rinsing and removing epibionts on the surface, the samples were placed in a blast drying oven (DHG-9070A, Shanghai, China) at 45 °C for 24 h until constant weight was achieved.

4.2. Alkaline Extraction Method

The alkaline extraction method for the five Eucheumatoid species was modified based on the method reported by Liu [28]. Briefly, 5.0 g of dried seaweed was cut into fragments of approximately 0.5 mm in length, followed by the addition of 100 mL of 7% KOH solution. The mixture was incubated at 70 °C for 75 min and then filtered through a 400-mesh nylon sieve (Solarbio, Beijing, China). The residual seaweed was rinsed with cold water and neutralized to neutral pH using 0.1 mol/L HCl. After adding 300 mL of deionized water, the mixture was extracted in a water bath at 85 °C for 2 h and filtered again with a 400-mesh nylon sieve. The filtrate was cooled and frozen at −20 °C for 1 day, after which the frozen gel strips were naturally thawed at room temperature for dehydration and rinsed three times with distilled water. Subsequently, the samples were soaked in absolute ethanol for 30 min and filtered through a 400-mesh filter cloth, and the ethanol-precipitated products were collected. The collected products were dried to constant weight in an oven at 40 °C, and the mass of the extracted carrageenan was measured using an electronic balance. The carrageenan yield, defined as the mass ratio of the extracted carrageenan to the dried seaweed sample, was calculated using the following formula:

$$\text{Yield } (\%) = \text{m2/m1} \times 100\%$$

m1 = mass of dried seaweed sample (g); m2 = mass of extracted carrageenan (g).

4.3. Ultrasonic-Assisted Enzymatic Extraction of Carrageenan

The ultrasonic-assisted enzymatic extraction procedure for carrageenan was modified on the basis of the method described by Xie Limei et al. [29]. Briefly, 5.0 g of dried seaweed was cut into fragments of approximately 0.5 mm, then treated with 8% KOH solution in a water bath at 75 °C for 3.5 h. After pretreatment, the alkaline solution was discarded, and the seaweed residues were rinsed to neutral pH for subsequent use. Deionized water was added

to the pretreated seaweed at a solid–liquid ratio of 1:30 (g/mL). The pH value was adjusted to 6.0 with citric acid, followed by the addition of 0.5% cellulase, 1.25% hemicellulase and 0.2% papain. The mixture was enzymatically hydrolyzed in a water bath at 60 °C for 2 h, and the enzyme activity was terminated by heating at 90 °C for 10 min. The inactivated sample was further treated with ultrasound at 30 °C and 350 W for 15 min, and finally boiled at 70 °C for 3 h. The mixture was filtered through a 400-mesh nylon gauze to remove seaweed residues. After the filtrate was cooled to approximately 45 °C, 95% ethanol was added at a volume ratio of 4:1 for alcohol precipitation. The precipitate was dried in a forced-air drying oven (DHG-9070A, Shanghai, China) at 40 °C and pulverized to obtain the final carrageenan product. The calculation formula for carrageenan yield was consistent with that described in Section 2.2.

4.4. Fourier-Transform Infrared Spectroscopy Analysis

The potassium bromide (KBr) pellet method was adopted for FTIR measurement. Briefly, 0.01 g of freeze-dried sample was mixed with KBr at a mass ratio of 1:100 and ground thoroughly into a homogeneous particle-free powder. The fine powder was transferred into a mold and compressed into a pellet under vacuum. The prepared pellet was immediately scanned using a Fourier-transform infrared spectrometer with a resolution of 4 cm^{-1} and a scanning range of 400–4000 cm^{-1} [30]. Three biological replicates were measured for each treatment ($n = 3$).

4.5. Gel Strength

A total of 100 mL of 3% (w/v) carrageenan solution containing 0.2% $CaCl_2$ was prepared. Briefly, 3.0 g of carrageenan was dissolved in 97.0 mL of deionized water, followed by the addition of 0.2 g of $CaCl_2$. The solution was poured into a Petri dish and kept standing overnight at 20 °C. Gel strength was determined within 20 s using a gel texture analyzer with a P/0.5R probe (TA. XT Plus, Stable Micro Systems, London, UK). The pretest speed and test speed were both set at 0.5 mm/s, the post-test speed was 1 mm/s, the trigger force was 5 g, and the compression distance was 20 mm. The maximum force at gel fracture was recorded in grams, and the gel strength was converted into g/cm^2 according to the sectional area of the probe. All measurements were performed in triplicate, and the average value with standard deviation was calculated [31]. The gel strength was calculated as follows:

$$\text{Gel strength } (g/cm^2) = F/S$$

F = pressure mass (g) at the moment the probe breaks the gel; S = cross sectional area of the plunger (cm^2).

4.6. Viscosity

We accurately weighed 1.5 g of carrageenan and dissolved it in 99 mL of deionized water. The mixture was then heated and dissolved in a water bath at 80 °C, then incubated at 75 °C for 15 min to prepare a 1.5% (w/v) carrageenan solution. The viscosity of the carrageenan solution was determined at a constant temperature of 75 °C using an NDJ-1 rotational viscometer, referring to the national standard GB 1886.169–2016 National Food Safety Standard—Food Additive: Carrageenan [32]. Three biological replicates ($n = 3$) were measured for each treatment.

4.7. Sulfate Content

Exactly 0.05 g of carrageenan sample was added to 50 mL of 1 mol/L HCl solution, and digested in a water bath at 100 °C for 5 h. After cooling to room temperature, activated carbon was added for decolorization. The mixture was then filtered to obtain the sample

digestive solution. Potassium sulfate (K_2SO4) was dried at 105 °C, and 0.1088 g was accurately weighed and dissolved in 1 mol/L HCl, then made up to a constant volume of 100 mL for standby storage. The K_2SO4 standard solution was diluted 5-fold. Subsequently, aliquots of 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the diluted standard solution were transferred into test tubes. The total volume of each tube was supplemented to 1 mL with 1 mol/L HCl. Each tube was mixed thoroughly with 3 mL of gelatin barium chloride solution and allowed to stand for 10 min. The absorbance was measured at 360 nm to establish the standard curve of sulfate concentration versus absorbance. Finally, 1 mL digestive solution was mixed with 3 mL of gelatin barium chloride solution and kept stationary for 10 min. The absorbance was determined using a UV–visible spectrophotometer at 360 nm, and the sulfate content of the sample was calculated according to the established standard curve [24]. Three biological replicates ($n = 3$) were measured for each treatment.

4.8. Textural Property Analysis

The texture analyzer (TA. XT Plus, Stable Micro Systems, London, UK), equipped with a standard probe, was calibrated for force and displacement before testing. The parameters of texture profile analysis, including probe type, compression ratio, test speed, interval between two compressions and trigger force, were set in advance. The formed gels were cut into cylindrical specimens and placed centrally on the texture analyzer platform to ensure uniform stress distribution. The program was initiated, and the probe vertically compressed the gel. After reaching the preset compression depth, the probe returned upward, followed by a short pause and a second compression to simulate mastication. Five textural properties (hardness, chewiness, cohesiveness, springiness and resilience) were recorded automatically during the test. Three biological replicates were measured for each treatment.

4.9. Statistical Analysis

FTIR and textural property analysis were plotted using Origin 2024 software, and statistical analyses of all data were performed with SPSS 26.0. Levene's test was used to verify the homogeneity of variances, and the Shapiro–Wilk test was applied to check the normality of residuals. Two-way ANOVA was performed separately for two factorial designs: algal species × extraction method, and algal species × salinity. When a significant interaction was detected between factors, simple effect analyses with Bonferroni correction based on estimated marginal means were carried out. For the algal species × extraction method model, pairwise comparisons were made between the two extraction methods within each algal species and among algal species under the same extraction method. For the algal species × salinity model, pairwise comparisons were conducted across salinity levels within each algal species and among algal species exposed to the same salinity. Statistical significance was defined as $p < 0.05$. All data are presented as mean ± standard deviation (SD).

5. Conclusions

This study conducted two independent two-way interaction analyses to demonstrated that algal species, salinity, extraction method, and their interactions significantly modulated the carrageenan profiles of five commercial Eucheumatoids ($p < 0.05$). Alkaline extraction yielded superior gel strength, viscosity and textural parameters, while the sulfate content was lower. Alkaline extraction was more suitable for Hn-G and Hn-R, whereas enzymatic extraction achieved higher carrageenan yields for Ma-Ks, Ma-R and Hn-E. Salinity exerted significant effects on gel strength and textural parameters, which peaked at 24‰ and declined at 18‰. Among all tested species, Hn-E exhibited the best comprehensive carrageenan

quality under different extraction protocols and salinity treatments. Nevertheless, Ma-R achieved the highest carrageenan yield and sulfate content at 18‰. FTIR spectra verified that κ-type carrageenan was present in all samples and no λ-type carrageenan signals were detected. Distinctive characteristic peaks of ι-type carrageenan were observed in alkali-extracted Hn-R and enzyme-assisted extracted Hn-E. The carrageenan type was strongly modulated by extraction methods, suggesting that extraction methods should be selected in a species-targeted manner. This study provides a theoretical basis for salinity management in Eucheumatoids cultivation, high-quality carrageenan extraction and optimization of processing technologies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse14212073/s1, Table S1: Levene's test for equality of error variances on yield of gel of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S2: Two-Way ANOVA of Species and extraction method on yield of gel of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S3: Levene's test for equality of error variances on gel strength of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S4: Two-Way ANOVA of Species and extraction method on gel strengthof carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S5: Levene's test for equality of error variances on sulfate content of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S6: Two-Way ANOVA of Species and extraction method on sulfate content of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S7: Levene's test for equality of error variances on viscosity of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S8: Two-Way ANOVA of Species and extraction method on Viscosity of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S9: Levene's test for equality of error variances on hardness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S10: Two-Way ANOVA of Species and extraction method on hardness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S11: Levene's test for equality of error variances on chewiness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S12: Two-Way ANOVA of Species and extraction method on chewiness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S13: Levene's test for equality of error variances on cohesiveness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S14: Two-Way ANOVA of Species and extraction method on cohesiveness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S15: Levene's test for equality of error variances on springiness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S16: Two-Way ANOVA of Species and extraction method on springiness of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S17: Levene's test for equality of error variances on resilience of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S18: Two-Way ANOVA of Species and extraction method on resilience of carrageenan prepared by alkali extraction and ultrasound-assisted enzymatic extraction from five Eucheumatoid Species; Table S19: Levene's test for equality of error variances on yield of gelof carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S20: Two-Way ANOVA of Species and salinity on yield of gel of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S21: Levene's test for equality of error variances on gel strength of carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S22: Two-Way ANOVA of Species and salinity on gel strength of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S23: Levene's test for equality of

error variances on sulfate contentof carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S24: Two-Way ANOVA of Species and salinity on sulfate content of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S25: Levene's test for equality of error variances on viscosity of carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S26: Two-Way ANOVA of Species and salinity on viscosity of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S27: Levene's test for equality of error variances on hardness of carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S28: Two-Way ANOVA of Species and salinity on hardness of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S29: Levene's test for equality of error variances on chewiness of carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S30: Two-Way ANOVA of Species and salinity on chewiness of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S31: Levene's test for equality of error variances on cohesiveness of carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S32: Two-Way ANOVA of Species and salinity on cohesiveness of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S33: Levene's test for equality of error variances on springiness of carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S34: Two-Way ANOVA of Species and salinity on springiness of carrageenan from five Eucheumatoid Species under three salinity gradients; Table S35: Levene's test for equality of error variances on resilience of carrageenan extracted from five Eucheumatoid seaweed Species under three salinity gradients; Table S36: Two-Way ANOVA of Species and salinity on resilience of carrageenan from five Eucheumatoid Species under three salinity gradients.

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Abbreviations

The following abbreviations are used in this manuscript:

K. alvarezii	*Kappaphycus alvarezii*
E. denticulatum	*Eucheuma denticulatum*
K. striatus,	*Kappaphycus striatus*
Hn-G	Hainan green *Kappaphycus alvarezii*
Hn-R	Hainan red *Kappaphycus alvarezii*
Ma-R	Malaysia red *Kappaphycus alvarezii*
Ma-Ks	Malaysia *Kappaphycus striatum*
Hn-E	Hainan *Eucheuma denticulatum*
AE	alkaline extraction
UAEE	ultrasound-assisted enzymatic extraction
TPA	textural parameter analysis
FTIR	Fourier-transform Infrared Spectroscopy
SOD	superoxide dismutase

APX	ascorbate peroxidase
GPX	glutathione peroxidase

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