

Identification of Properties and Structure of ι-Carrageenan Prepared with Different Alkali Treatment Times

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Abstract

This study systematically investigated the influence of alkaline treatment duration (2–6 h) on the structural characteristics and functional properties of ι-carrageenan derived from *Eucheuma denticulatum*. The results indicated that a 3 h alkaline treatment achieved the highest yield (46.6%) and significantly enhanced gel strength by facilitating the formation of 3,6-anhydrogalactose (3,6-AG). In contrast, prolonged treatment reduced both molecular weight and yield. Moderate alkaline treatment (3–4 h) improved crystallinity and thermal stability while maintaining water-holding capacity above 80% after freeze-thaw cycles. FT-IR and XRD analyses confirmed that alkaline treatment preserved the fundamental structure of ι-carrageenan while altering molecular packing order. These findings provide valuable guidance for industrial production, indicating that a 3–4 h alkaline treatment not only optimizes yield but also enhances functional performance. Overall, the study elucidated how alkaline treatment time modulates ι-carrageenan quality through 3,6-AG formation, molecular chain degradation, and crystalline reorganization, thereby offering theoretical guidance for optimizing food-grade carrageenan production.

1. Introduction

Carrageenan (CGN) is a primary polysaccharide derived from the extracellular matrix of red algae (such as *Eucheuma*, *Gigartina*, *Chondrus*, and *Hypnea*), which is widely used in the food, cosmetics, and pharmaceutical industries [1]. The number and position of sulfate groups on the carrageenan disaccharide determine its type as κ-, ι-, and λ-carrageenan. The structural difference between κ-carrageenan and ι-carrageenan lies in their linear backbones: κ-carrageenan consists of α(1,3)-D-galactose-4-sulfate and β(1,4)-3,6-anhydro-D-galactose, while ι-carrageenan contains linear skeleton with alternating α(1,3)-D-galactose-4-sulfate and β(1,4)-3,6-anhydro-D-galactose-2-sulfate [2]. Compared to κ-carrageenan, ι-carrageenan is a more highly sulfated helical polysaccharide [3]. The elevated sulfate content confers upon ι-carrageenan gels a set of distinctive advantages, such as high elasticity, robust freeze-thaw resistance, and strong tolerance to high salinity environments. Additionally, ι-carrageenan exhibits various bioactivities such as antioxidant effects, antiviral properties, gastric mucosa protection, and wound healing promotion [4–7]. Natural carrageenan is widely used as a food additive to improve texture. Although the native form of carrageenan (200–800 kDa) has been declared harmless to humans, several studies have demonstrated that long-term feeding of rats with degraded ι-carrageenan (with average molecular weight ranging from 20 to 40 kDa) over a 24-month period may induce colitis, secondary metaplasia, and ultimately tumorigenesis [8]. European Union has set a detection limit of 5% for low-molecular-weight components (< 50 kDa) in carrageenan to ensure its safety [9]. Therefore, monitoring the molecular weight of carrageenan is of great significance to its production quality and safety.

The production process of carrageenan mainly entails alkali treatment to extract semi-refined carrageenan from seaweed, followed by filtration to eliminate residual components like cellulose, and ultimately precipitation with alcohol or salts for carrageenan recovery [10]. The alkali treatment,

encompassing alkali type, treatment duration, and alkali concentration, plays a crucial role in determining the final structural properties of the product. Previous studies have shown that prolonged low-temperature alkaline pretreatment of *M. stellatus* seaweed facilitated the extraction of biopolymers containing reduced amounts of biological carrageenan precursors (ν - and μ -monomers), meanwhile although the relative proportions of κ - and ι -monomers remained unchanged, the elasticity of the biopolymers is enhanced [11]. Additionally, during alkali extraction, ι -carrageenan molecules undergo a conformational transition from a random coil to a helical structure. This structural rearrangement significantly influences the gelling mechanism and rheological properties of ι -carrageenan [12]. Therefore, a systematic investigation into the impact of alkaline treatment time on the structural characteristics of ι -carrageenan, specifically its molecular weight and functional properties, is crucial for optimizing production processes and acquiring ι -carrageenan products with targeted performance.

This study aimed to investigate the effects of alkaline treatment time on the structural and functional properties of ι -carrageenan. Through adjusting the alkaline treatment time during extraction, key parameters of the resulting ι -carrageenan, including chemical composition (e.g., sulfate content), melting and gelling temperature, apparent viscosity, gel strength, and water-holding capacity, were evaluated. Meanwhile, the ι -carrageenan samples were characterized via fourier-transform infrared (FT-IR) spectroscopy, size-exclusion chromatography coupled with differential refractive index detection and multi-angle laser light scattering (SEC-DRI-MALLS), as well as thermogravimetric analysis (TGA). The expected findings can lay a theoretical foundation for optimizing the alkali process of ι -carrageenan, while also providing technical support for its applications in the food, pharmaceutical, and related industries.

2. Materials and methods

2.1. Materials

Eucheuma denticulatum was supplied by Greenfresh Zhang Zhou Foods Co., Ltd. (Zhangzhou, China). Analytical grade chemicals including sodium hydroxide (NaOH), potassium chloride (KCl), calcium chloride (CaCl_2), potassium sulfate (K_2SO_4), absolute ethanol, and sodium nitrate (NaNO_3) were procured from China National Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China). Chromatographic standards of D-galactose and D-galacturonic acid were obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China).

2.2 Preparation of ι -carrageenan

Weighed 15 g of the washed dry *Eucheuma denticulatum*, added an alkaline solution containing 10% NaOH and 12% KCl at a solid to liquid ratio of 1:30 (w:v). The mixture of *Eucheuma denticulatum* and alkaline solution was heated at 65°C for 2, 3, 4, 5 and 6 h, respectively. Subsequently, rinsed *Eucheuma denticulatum* multiple times until it reached a neutral pH, added a 0.15% HCl at a solid to liquid ratio of 1:30 (w:v) for 15 min of acidification treatment. After acidification, the sample was rinsed thoroughly until the pH reached neutrality. Finally, added distilled water at a solid to liquid ratio of

1:30 (w:v), and then boiled at 100°C for 3 h to gain carrageenan gel. Filtered the hot gel solution, subjected it to vacuum freeze drying, and then ground it into powder. The prepared carrageenans from *Eucheuma denticulatum* with alkaline solution for 2, 3, 4, 5 and 6 h, are designated as ι-2, ι-3, ι-4, ι-5, and ι-6, respectively.

2.3 Determination of ι-carrageenan yield

The yield of ι-carrageenan was determined by the ratio of the weight of dried raw materials of *Eucheuma denticulatum* (W_0) to the weight (W_1) of the final ι-carrageenan product.

$$\text{Yield (\%)} = \frac{W_1}{W_0} \times 100$$

2.4 Determination of physicochemical properties of ι-carrageenan

Total sugar content of ι-carrageenan was detected through phenol-sulfuric acid method. The sulfate and 3,6-AG contents of ι-carrageenan were determined using the BaCl_2 turbidimetric [13] and resorcinol methods[14], respectively. Whiteness of ι-carrageenan was measured using the ADCL-WSI Whiteness colorimeter (ADCL-WSI, Beijing Chentaikete Instrument Technology Co., LTD., Beijing, China).

2.4.1 Gel properties of ι-carrageenan

The gel strength of ι-carrageenan was determined following the previous method [15] using a texture analyzer (TA-XTplus, Waters Instruments Co., Ltd., Milford, MA, USA). Briefly, 1.5% (w/v) ι-carrageenan solution containing 0.2% (w/v) CaCl_2 was prepared, and heated in a boiling water bath until complete dissolution. Subsequently, 10 mL of the ι-carrageenan solution was aliquoted into each cylindrical mold, followed by cooling and solidification at room temperature for 12 h. The P5/S probe was employed, and the test conditions was as following: pre-test speed of 2 mm/s, test speed of 1 mm/s, post-test speed of 2 mm/s, and trigger force of 3 g.

2.4.2 Melting and gelling temperature of ι-carrageenan

Following a previous method [16] with modifications, ι-carrageenan solution of 1.5% (w:v) was prepared with 0.2% CaCl_2 . After cooling and solidification, glass beads were placed on the surface of ι-carrageenan gel. The temperature was raised at a rate of 1 °C/min, and the melting temperature was recorded when the glass beads fell to the bottom of the test tube. The test tube was tilted up and down until the glass beads at the bottom stopped moving, of which the temperature was recorded as the gelling temperature.

2.5 Detection of apparent viscosity of ι-carrageenan

The apparent viscosity of ι-carrageenan was determined using an Anton Paar MCR 302 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a parallel plate geometry (50 mm diameter, 1 mm gap). The measurements were performed using a PP50 measuring system with the plate temperature maintained at 75°C. The shear rate was linearly ranged from 0.1 s⁻¹ to 600 s⁻¹ to record the flow curve of the ι-carrageenan solution.

2.6 Determination of molecular weight of ι-carrageenan

Molecular weight determination of ι-carrageenan was performed using a multi - angle laser light scattering gel permeation chromatography system equipped with a differential refractive index detector (SEC-DRI-MALS, Wyatt technology, CA, USA), according to the previous method reported [17] with modifications. The chromatographic separation was performed using a tandem column system consisting of TSK gel G4000PWXL and TSK gel G2500PWXL connected in series. The mobile phase consisted of 0.1 M NaNO₃ containing 0.035% (v/v) ProClin™ 300, delivered at a flow rate of 0.60 mL/min. The column compartment and detector were both maintained at 35°C. The ι-carrageenan samples were prepared at a concentration of 1 mg/mL in the mobile phase, filtered through a 0.22 μm aqueous membrane filter, and injected into the detected system at a volume of 100 μL. The total run time was set as 60 min.

2.7 Analysis of water-holding capacity (WHC) of ι-carrageenan

A 10 mL ι-carrageenan solution (1.5% w/v; containing 0.2% CaCl₂) was added to a centrifuge tube, stored at 4°C for 24 h, and then transferred to -20°C freezing for another 24 h. After a 6 h thawing process, the ι-carrageenan sample was centrifuged at 10,000 ×g for 15 min. Excess water was removed using filter paper, and the residual weight was recorded [18]. WHC was calculated using the following formula:

$$\text{WHC (\%)} = \frac{W_n}{W_0} \times 100$$

where W_0 is the original weight of the gel per tube; W_n is the residual weight of the gel per tube after draining the separated water.

2.8 Low-Field nuclear magnetic resonance (LF-NMR) analysis of ι-carrageenan

Moisture migration in the ι-carrageenan samples was analyzed using a 23 MHz low-field nuclear magnetic resonance analyzer equipped with an imaging system (Model HT-MRSI20-60A, Shanghai Huan Tong Science & Education Equipment Co., Ltd., China). Each 3 mL 1.5% (w/v) ι-carrageenan solution containing 0.2% CaCl₂ was placed into a 5 mL cylindrical cryovial. After gelation, transverse relaxation curves (T_2) of ι-carrageenan gel were acquired using the Carr-Purcell-Meiboom-Gill (CPMG) pulse

sequence. The echo time, number of echoes, and number of scans were set as 3.0 ms, 2048, and 5, respectively.

2.9 Fourier Transform Infrared (FT-IR) spectroscopy of ι-carrageenan

Dried ι-carrageenan powder was mixed with dried potassium bromide (KBr) at a ratio of 1:100 (w/w) and pressed into pellets. FT-IR spectra of ι-carrageenan were recorded by using an IS50 FT-IR spectrometer (Thermo Fisher Scientific Inc., USA) at the wavenumbers of 4000–400 cm^{-1} with 32 scans per spectrum [19].

2.10 Monosaccharide composition determination of ι-carrageenan

Monosaccharide composition of ι-carrageenan was determined via PMP derivatization [20]. Briefly, 5 mg oven-dried (to constant weight) ι-carrageenan sample was hydrolyzed with 1 mL 2 M trifluoroacetic acid (TFA) at 120°C for 4 h. Residual TFA was removed by nitrogen-blow after adding methanol. The dried ι-carrageenan sample was redissolved in 400 μL ammonia solution and 400 μL 0.3 M PMP-methanol, and then incubated at 70°C for 30 min. Excess ammonia was evaporated under nitrogen, with traces removed by washing with methanol three times. Subsequently, 1% acetic acid and 1 mL chloroform were added; after vortexing, the aqueous phase was extracted, filtered through a 0.22 μm membrane, and analyzed by HPLC (LC-20A system, Shimadzu Corporation, Japan) on a 150 $\times$ 3.0 mm column (10 μm particle size). The mobile phase was 5% acetonitrile (20 mM ammonium acetate, solvent A) and acetonitrile (solvent B) at 87:13 (A:B), with a flow rate of 0.6 mL/min and injection volume of 20 μL .

2.11 Thermogravimetric analysis (TGA) of ι-carrageenan

The thermal stability of ι-carrageenan was investigated by thermogravimetric analysis (TGA8000, PerkinElmer, Waltham, MA, USA). Approximately 3–6 mg of ι-carrageenan powder was placed in an alumina crucible and heated from 40°C to 600°C at a rate of 10 °C/min under a nitrogen purge gas flow of 20 mL/min. The mass loss and its first derivative (DTG) were recorded during the heating process.

2.12 X-ray Diffraction (XRD) analysis of ι-carrageenan

Crystallinity of the ι-carrageenan samples was analyzed by X-ray diffraction (Bruker D8 Venture, Karlsruhe, Germany) using Cu K α radiation. XRD analysis were performed over a 2 θ range of 5° to 50° at a rate of 5°/min [21].

2.13 Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD). Analysis of variance was performed using IBM SPSS Statistics (SPSS 19 for Windows). Tukey's multiple comparison test was applied, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Effect of alkaline treatment time on yield and chemical composition of ι-carrageenan

The effect of alkaline treatment time on the yield of ι-carrageenan is shown in **Fig. 1**. The yield of ι-carrageenan exhibited a trend of first increasing and then decreasing with the extension of alkaline treatment time, and the maximum yield of 46.6% achieved at an alkaline treatment time of 3 h. This phenomenon may be attributed to the fact that prolonged alkaline treatment can enhance the disruption of cell walls in *Eucheuma denticulatum*, thereby facilitating the extraction of ι-carrageenan molecules. However, excessive treatment duration may cause part of the gelatinous solution to dissolve in the alkaline liquor, which is subsequently lost during the rinsing process.

The chemical composition of ι-carrageenan extracted with different alkaline treatment time, including total sugar content, sulfate content and 3,6-AG content were analyzed (**Table 1**). The results showed that prolonged alkali treatment time exerted no significant effect on the total sugar content and sulfate content of ι-carrageenan. The absence of variation in total sugar content may be related to the limitations of the detection method, as the phenol–sulfuric acid assay reports values in glucose-equivalent concentrations, which may not accurately reflect complex carbohydrates that are not simple glucose polymers [22]. Similarly, the stability of sulfate groups in ι-carrageenan under alkaline conditions likely accounts for their resistance to hydrolysis or desulfation. Previous studies have also indicated that the specific positioning and bonding configuration of sulfate groups in ι-carrageenan contribute to their enhanced alkali tolerance [23]. The 3,6-AG content of ι-carrageenan initially increased and subsequently decreased with extended alkali treatment time, reaching optimal levels after 3-4 hours of treatment. This pattern suggested that moderate alkali treatment facilitates the structural conversion of D-galactose-6-sulfate residues linked by 1,4-glycosidic bonds, wherein the elimination of C₆ sulfate groups promotes the formation of 3,6-anhydro-D-galactose units. This transformation mechanism has been reported to significantly improve gel strength of ι-carrageenan [24].

Table 1: The chemical composition and whiteness of ι-carageenan

sample	Total sugar content (%)	Sulfate content (%)	3,6-AG content (%)	Whiteness (%)
ι-2	61.05±1.14 ^a	16.58±0.50 ^a	21.12±0.47 ^b	45.38±0.02 ^d
ι-3	58.94±1.43 ^a	16.01±0.04 ^a	22.05±0.13 ^a	48.64±0.26 ^c
ι-4	61.43±2.46 ^a	15.99±0.16 ^a	22.73±0.34 ^a	53.28±0.17 ^a
ι-5	58.32±0.38 ^a	16.17±0.22 ^a	21.10±0.27 ^b	50.70±0.06 ^b
ι-6	61.10±1.21 ^a	16.36±0.24 ^a	20.41±0.51 ^b	50.82±0.13 ^b

a, b, c and d indicate significant differences (*p* < 0.05).

3.2 Effect of alkaline treatment time on whiteness of ι-carrageenan

The whiteness of ι-carrageenan reflects the degree of pigment removal during extraction, and higher whiteness directly affects the better quality, performance, and applications of its product. The results demonstrated that prolonged alkali treatment time significantly enhanced whiteness of ι-carrageenan, with the maximum whiteness value of 53.28% achieved after 4 hours of alkali treatment (**Table 1**). This finding may be attributed to the fact that during alkaline treatment, algal cells are disrupted, and the intracellular pigments, primarily water-soluble phycobilins, including phycoerythrin and phycocyanin, are prone to photolysis, thereby removing greater quantity of pigments and enhancing the whiteness of ι-carrageenan [16].

3.3 Effect of alkaline treatment time on gel strength of ι-carrageenan

Gel strength serves as a critical indicator for evaluating carrageenan quality. As shown in **Fig. 2**, the gel strength of ι-carrageenan displayed a distinct trend of initial increase followed by a decrease with the extension of alkali treatment duration. The results revealed that ι-carrageenan extracted with 3 hours alkali treatment demonstrated much higher gel strength than that of one with 2 hours alkali treatment. This enhancement may be attributed to the gradual disruption of algal cell structures by sodium hydroxide, which facilitates the formation of 3,6-anhydrogalactose through sulfate group elimination, thereby improving gelling properties. Studies have demonstrated a strong positive correlation between gel strength and 3,6-AG content, where higher 3,6-AG levels correspond to greater gel strength [25]. Conversely, gel strength shows a negative correlation with sulfate content. This inverse relationship stems from the electrostatic repulsion generated by negatively charged sulfate groups between carrageenan molecules, which hinders their close association and aggregation, thereby impeding the formation of compact gel networks. As sulfate content decreases, the reduced electrostatic repulsion allows more effective intermolecular interactions, facilitating the development of denser gel structures with enhanced gel strength [26]. Thus, a slight decrease in sulfate content for ι-carrageenan extracted with 3 hours alkali treatment, may also contribute to its higher gel strength. However, no statistically significant differences in gel strength were observed among ι-carrageenan with 2–4 hours alkali treatment. This phenomenon may be attributed to the inherently soft texture and high elasticity of ι-carrageenan, which could result in insufficient sensitivity in texture analyzer measurements, thereby making it challenging to distinguish subtle variations between these ι-carrageenan samples. However, when the alkali treatment time is prolonged beyond 5-6 hours, it leads to a significant reduction in the gel strength of ι-carrageenan. This deterioration may be caused by either insufficient conversion due to suboptimal treatment intensity (concentration and duration) or excessive processing conditions. Furthermore, the presence of substantial precursor units in seaweed has been reported to adversely affect gel properties [27]. In addition, as described above, the reduced 3,6-AG content and the slight increased sulfate content may result in the reduced gel strength of ι-carrageenan.

3.4 Effect of alkaline treatment time on melting and gelling temperatures of ι-carrageenan

The melting temperature of carrageenan gel reflects the energy required to disrupt its three-dimensional network, while the gelling temperature influences its interaction with other components and ultimately determines its application performance. As shown in **Fig. 3**, with alkaline treatment time increased, the melting and gelling temperatures of ι-carrageenan was decreased, where ι-4 exhibited low gelling and melting temperatures of 58.67°C and 73°C, respectively. The observed melting temperature > gelling temperature represents a typical thermal hysteresis phenomenon common to carrageenan (and polysaccharide gels in general). In addition, the thermal behavior suggested that complete dissolution can be achieved at processing temperatures exceeding 73°C, significantly lower than other gelling agents like agar (requiring > 95°C for complete melting) [28], thereby offering considerable energy-saving advantages in industrial processing.

3.5 Effect of alkaline treatment time on apparent viscosity of ι-carrageenan

The steady-shear behavior of ι-carrageenan extracted with different alkaline treatment time was determined within shear rate range of 0.1–600 s⁻¹. As shown in **Fig. 4**, all flow curves of ι-carrageenan exhibited that apparent viscosity decreased with increasing shear rate, indicating that these ι-carrageenan samples exhibited typical non-Newtonian fluid behavior and shear-thinning characteristics [29]. The decrease in the apparent viscosity of ι-carrageenan can be attributed to the fact that under low shear conditions, ι-carrageenan forms a dense network structure via covalent bonding, resulting in a high-viscosity gel with relatively stable apparent viscosity. However, as the shear rate increased, the applied shear force disrupts these entanglement points, leading to the disintegration of the gel network, in turn cause a decrease in apparent viscosity [30]. It was observed that the viscosity levels and the extent of variation differed with the alkaline treatment time. Samples ι-2, ι-5, and ι-6 exhibited higher initial viscosities, whereas samples ι-3 and ι-4 showed lower initial viscosities and displayed relatively less pronounced shear-thinning behavior. This could be attributed to the fact that the optimal alkaline treatment time of 3-4 hours facilitating the formation of more 3,6-AG, which enhances molecular chain regularity.

3.6 Effect of alkaline treatment time on molecular weight of ι-carrageenan

The molecular weight of ι-carrageenan extracted with different alkaline treatment time was analyzed (**Fig. 5**). The results revealed that alkali extracted carrageenan exhibited broad chromatographic peaks with multiple shoulders, accompanied by polydispersity indices (M_w/M_n) exceeding one, indicating the heterogeneous nature of the extracted ι-carrageenan. Notably, prolonged alkali treatment time led to progressive molecular weight reduction. This phenomenon likely originates from alkaline-induced cleavage of glycosidic bonds within the carrageenan backbone, resulting in significant polymer degradation. This observation aligns with previous findings demonstrated that hydroxyl ions (OH⁻) can disrupt hydrogen bonds and other covalent linkages in algal cell walls, ultimately causing polysaccharide depolymerization [31]. In accordance with EU regulations stipulating that enteropathogenic low-molecular-weight carrageenan fractions (<50 kDa) was less than 5% of the final product [32]. However, when quantitatively analyzed sub-50 kDa fractions, all ι-carrageenan samples contained < 50 kDa

components exceeding 5% as shown in **Table 2**. Furthermore, the relative content of < 50 kDa fragments exhibited a positive correlation with alkaline treatment time, implying that reduction of alkaline treat time could be applied for minimizing low-molecular-weight fractions.

Table 2: The Molecular weight variation of ι-carrageenan

sample	Mw/kDa	Proportion(%)	Mw/kDa	Proportion(%)
ι-2	987.8±29.7	94.9±2.3	52.7±3.5	5.1±2.3
ι-3	905.4±8.1	95.0±1.7	54.8±1.6	5.0±1.5
ι-4	759.4±10.0	94.0±1.7	51.8±1.7	6.0±1.7
ι-5	843.3±48.7	93.9±2.9	51.5±1.2	6.1±2.9
ι-6	782.8±10.6	93.9±0.3	51.4±2.1	6.1±0.3

3.7 Effect of alkaline treatment time on water holding capacity of ι-carrageenan

To evaluate water-holding capacity of ι-carrageenan affected by different alkaline treatment time, weight loss of ι-carrageenan gel after freeze-thaw cycling was measured. As illustrated in **Fig 6**, all ι-carrageenan samples maintained high water-holding capacity exceeding 80% following a single freeze-thaw cycle. This remarkable performance can be attributed to the unique double-helical structure of ι-carrageenan, which effectively restricts ice crystal growth during freezing. However, ι-3 displayed the lowest water-holding capacity among all tested samples, while no statistically significant differences were observed between other samples ($p > 0.05$). This is because gels produced ice crystals internally after freezing and thawing, which destroyed the intermolecular structure, molecules aggregated into clusters, water was lost with a decrease in water-holding capacity, and the gel strength increased [33]. Moreover, this observation is consistent with the aforementioned variations in gel strength, suggesting a structure-property relationship between the molecular organization and functional performance of ι-carrageenan.

LF-NMR technology is commonly used to analyze the moisture distribution and migration changes in gel samples through the relaxation time of hydrogen protons [34, 35]. The transverse relaxation time indicates the mobility of water molecules, where the different peak curves of T_2 represent different states of water molecules, such as T_{21} (10-100 ms) means immobilized water and T_{22} (100-1000 ms) is free water [36]. As shown in **Fig. 7**, ι-carrageenan showed two peak areas of T_{21} and T_{22} at 10-100 ms and 100-1000 ms, respectively, indicated the presence of immobilized water and free water. With the extension of alkali treatment time, T_{22} showed a tendency to shift to the right, indicating that ι-carrageenan has a weak binding ability with water, and the immobilized water in the molecules is transformed into free water. This phenomenon may be attributed to the destruction of ι-carrageenan molecular chains during the alkali treatment, which increases the mobility of free water in the three-dimensional network structure of the gel. As shown in **Fig. 7**, the proportion of P_{22} first increased and then decreased as follows: ι-2 (91.15%), ι-3 (97.70%), ι-4 (97.87%), ι-5 (97.43%), and ι-6 (97.29%); the

change trend of P_{21} was opposite to that of P_{22} . This may be because the exposure of hydroxyl groups increases with the extension of alkali treatment time, providing binding sites for the combination of free water with ι -3 and ι -4 molecules. However, the subsequent decrease in P_{21} may be due to the molecular chain breakage caused by the prolonged alkali treatment time, which enhances the flexibility of ι -carrageenan molecular chains, leads to less free water and more immobilized water in the three-dimensional network structure of the gel. These results are consistent with the changes in moisture distribution of mechanically activated κ -carrageenan reported previously [37].

3.8 Effect of alkaline treatment time on FT-IR spectra of ι -carrageenan

Fig. 8 presents the FT-IR spectra of ι -carrageenan extracted with different alkaline treatment time. All ι -carrageenan samples exhibited characteristic absorption peaks at 3440 cm^{-1} , 2920 cm^{-1} , 1640 cm^{-1} , 1069 cm^{-1} , 930 cm^{-1} , 805 cm^{-1} , and 845 cm^{-1} . It was suggested that prolonged alkali treatment time did not disrupt the repeating units or primary structure of ι -carrageenan. However, prolonged alkali treatment time could influenced FT-IR peak intensities of ι -carrageenan. The broad peak at 3440 cm^{-1} was attributed to O-H stretching vibrations, which showed varying intensities with alkali treatment time. The highest peak intensities at 3440 cm^{-1} was observed for ι -4. The increased hydroxyl peak intensity in ι -4 may result from molecular chain scission during alkali treatment, which exposes previously encapsulated hydroxyl groups and enhances their interaction with free water molecules—thereby improving hydrophilicity [38]. The peaks at 2920 cm^{-1} and 1640 cm^{-1} were caused by the stretching vibrations of C-H and bound water, respectively. Similarly, ι -4 also displayed the highest peak intensity in 1640 cm^{-1} . This trend correlated with the moisture distribution results, where ι -4 exhibited a higher P_{22} value than ι -3. Besides, the absorptions at 1069 cm^{-1} and 930 cm^{-1} , corresponding to C-O bonds of 3,6-AG, which showed the highest intensities for ι -4, consistent with its highest 3,6-AG content as listed in **Table 1**. The band at 1250 cm^{-1} , arising from asymmetric stretching of sulfate groups, confirmed the presence of sulfate groups in ι -carrageenan. Additionally, the characteristic peaks at 805 cm^{-1} and 845 cm^{-1} indicated sulfate substitution at C_2 and C_4 positions, respectively. The A805/A845 ratio remained approximately 1 across samples, suggesting sulfate ester substitution was insensitive to the tested alkaline treatment time, aligning with the non significant changes in sulfate content as shown in **Table 1** [39, 40].

3.9 Effect of alkaline treatment time on monosaccharide composition of ι -carrageenan

The effect of different alkaline treatment time on the monosaccharide composition of ι -carrageenan is presented in **Table 3**. The results indicated that all ι -carrageenan samples prepared with varying alkali treatment time contained four identical types of monosaccharides. Among them, galactose was the dominant monosaccharide, accompanied by glucose, and small amounts of galacturonic acid and xylose. Prolonging the alkali treatment time did not alter the types of monosaccharides, which was consistent with the previous findings that carrageenan degraded by light showed no changes in monosaccharide types but differed in their contents [21]. As shown in **Table 3**, the extension of alkali

treatment time led to a decrease in galactose content and a corresponding increase in galacturonic acid content for ι-carrageenan. This phenomenon may be attributed to the hydrolysis of partial galactose into galacturonic acid, or result from alkali treatment promoting the desulfation of precursor substances through cyclization reactions [41]. Compared with the ι-2 sample, the xylose content increased in ι-carrageenan samples treated with alkali for 3–6 h. This could be due to the destruction of hemicellulose structures in the algal cell wall during the high-temperature alkali extraction process, thereby introducing more xylose [42].

Table 3: Monosaccharide composition of ι-caragean

	Gal-A% [Ⓢ]	Glc% [Ⓢ]	Gal% [Ⓢ]	Xyl% [Ⓢ]
ι-2	2.60±0.53 ^{ab}	18.02±0.21 ^{ab}	77.90±0.00 ^a	1.47±0.74 ^b
ι-3	1.93±0.01 ^b	18.98±0.26 ^a	76.57±0.18 ^a	2.52±0.09 ^a
ι-4	2.46±0.80 ^{ab}	18.01±0.61 ^{ab}	76.94±1.32 ^a	2.59±0.09 ^a
ι-5	2.71±1.13 ^{ab}	17.59±0.50 ^b	77.58±1.58 ^a	2.12±0.05 ^{ab}
ι-6	3.38±0.04 ^a	18.48±0.10 ^{ab}	75.51±0.16 ^a	2.63±0.02 ^a

a and b indicate significant differences (*p* < 0.05).

3.10 Effect of alkaline treatment time on XRD pattern of ι-carrageenan

XRD analysis results of ι-carrageenan prepared with different alkali treatment time are shown in **Fig. 9**. The XRD patterns of all ι-carrageenan samples with different alkali treatment time were similar, along with a broad diffraction peak in the range of 15°–25°, indicating a semi-crystalline polysaccharide structure [43], which aligned with the inherent flexibility of carrageenan helical structure. The broad diffraction peak near 2θ = 22° was a characteristic feature of the amorphous structure of ι-carrageenan. With prolonged alkali treatment time, the positions of the main diffraction peaks and the corresponding interplanar spacings varied greatly among ι-carrageenan samples. Specifically, as the alkali treatment time increased, the main diffraction peaks of ι-carrageenan appeared at 21.44°, 21.16°, 21.40°, 22.12°, and 22.02°. Compared to ι-2, ι-3 exhibited peak shifts , possibly due to alkali treatment promoting the formation of 3,6-AG, thereby altering the charge distribution and spatial conformation of the molecular chains, which corresponded to the observed increase in gel strength. The peak position of ι-4 was close to that of ι-2, but with sharper peaks, suggesting improved crystallinity. Since a narrower peaks typically indicates more ordered crystal structures. In general, sharp and narrow peaks reflect crystalline structures, while broad peaks indicate amorphous domains [44]. Starting from ι-5, the main diffraction peaks shifted compared to those of the previous samples, and the smallest interplanar spacing was 0.402 nm and a decrease in crystallinity, reflecting changes in the molecular chain packing periodicity. This may be attributed to alkali-induced hydrolysis of some glycosidic bonds in the polysaccharide

chains, leading to further modifications in chain length and a restructuring of the crystalline arrangement. As reported previously [45], *Lycium barbarum* polysaccharides extracted using different processes varied in 2 θ angles and crystallite structures were observed, which likely due to the disruptive effects of harsh acid-base conditions on polysaccharide crystallinity. In summary, alkali treatment time influences the crystallinity and molecular packing of ι -carrageenan, with the sample treated for 4 hours (ι -4) exhibiting the highest crystallinity.

3.11 Effect of alkaline treatment time on TGA of ι -carrageenan

The mass loss process of ι -carrageenan can be divided into two stages as shown in **Fig. 10**. In the first stage (40–200°C), the weight loss was attributed to the evaporation of free water within the carrageenan molecules. In the second stage (200–500°C), the mass gradually decreased to approximately 40%, which may be associated with the degradation of carbohydrates [46]. Among ι -carrageenan extracted with different alkali treatment times, ι -2 exhibited the highest mass loss rate of 61.53%, while ι -3 had the lowest initial thermal degradation temperature of 225°C. In contrast, ι -4 demonstrated the highest initial thermal degradation temperature of 228.36°C and the lowest mass loss rate of 57.39%. In addition, within the range of 150–500°C, the thermal decomposition of ι -carrageenan led to an increase in mass loss. This was due to the degradation of ι -carrageenan, where hydroxyl groups of galactose undergo rapid dehydration and decomposition; C–H, C–O, and C–C bonds are broken; and the backbone is disrupted [25]. Among all ι -carrageenan samples, ι -4 showed the lowest mass loss rate, possibly because moderate alkali treatment promotes appropriate aggregation of polysaccharide molecules, facilitating their transition from a loose amorphous structure to a more compact crystalline or helical configuration, thereby enhancing thermal stability. This finding aligns with the results observed in alkali extracted mung bean peel polysaccharides, which exhibited higher thermal stability as compared to water-extracted polysaccharides [47].

4. Conclusions

This study investigated the effect of alkaline treatment time on the yield, physicochemical and functional characteristics of ι -carrageenan. The results demonstrated that an optimal alkaline treatment time of 3 hours achieved the highest yield (46.6%) while promoting the conversion of D-galactose-6-sulfate to 3,6-AG, which significantly enhancing the gel strength of ι -carrageenan. Prolonged treatment time (> 5 hours) resulted in molecular weight reduction and decreased yield. Rheological analysis confirmed shear-thinning behavior in all ι -carrageenan samples, where ι -carrageenan treated for 3–4 hours alkaline treatment exhibiting more stable viscosity characteristics. Additionally, extended alkaline treatment time increased free water mobility (T_{22} relaxation time), maintaining good water-holding capacity (> 80% after freeze-thaw cycles) and excellent thermal stability. FT-IR and XRD analyses showed that alkaline treatment time does not disrupt the fundamental structure of ι -carrageenan but influences its crystallinity and molecular arrangement. Overall, the findings provide critical insights for the industrial production of ι -carrageenan, demonstrating that 3–4 hours of alkaline treatment ensures optimal yield while maintaining superior gel performance and product quality.

Declarations

Author contribution

Jiawen Shi: Investigation, Writing-Original Draft. **Tao Hong:** Validation, Writing-Review & Editing. **Zhipeng Li:** Formal analysis, Validation. **Yujia Ou:** Formal analysis, Writing-Review & Editing. **Yanbing Zhu:** Conceptualization, Formal analysis. **Yuanfan Yang:** Formal analysis, Validation, Writing-Review & Editing. **Zedong Jiang:** Conceptualization, Writing-Review & Editing. **Hui Ni & Mingjing Zheng:** Writing-Review & Editing, Supervision, Funding acquisition.

Conflict of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Figures

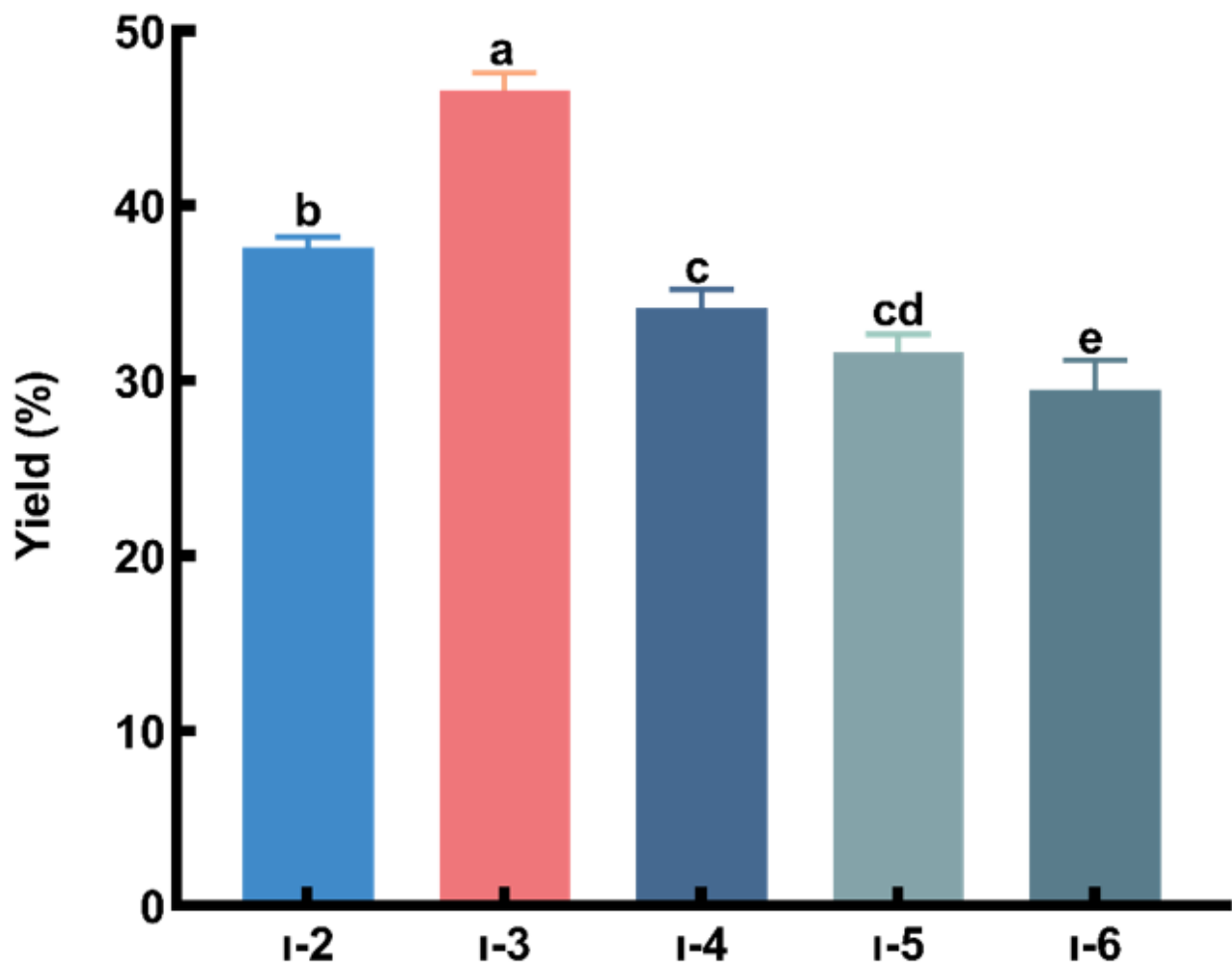


Figure 1

Yield of ι-Carrageenan

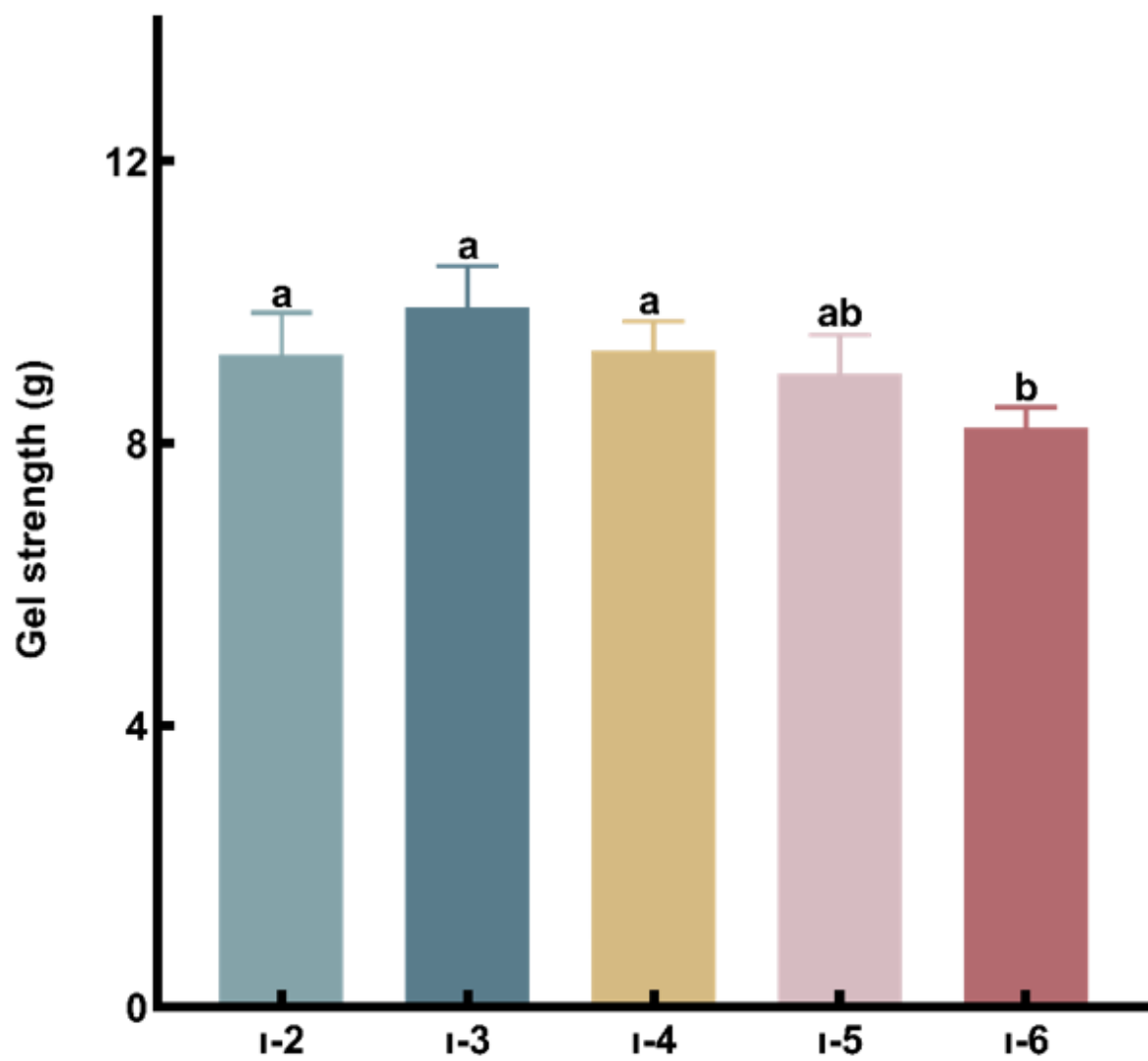


Figure 2

Gel strength of ι-Carrageenan

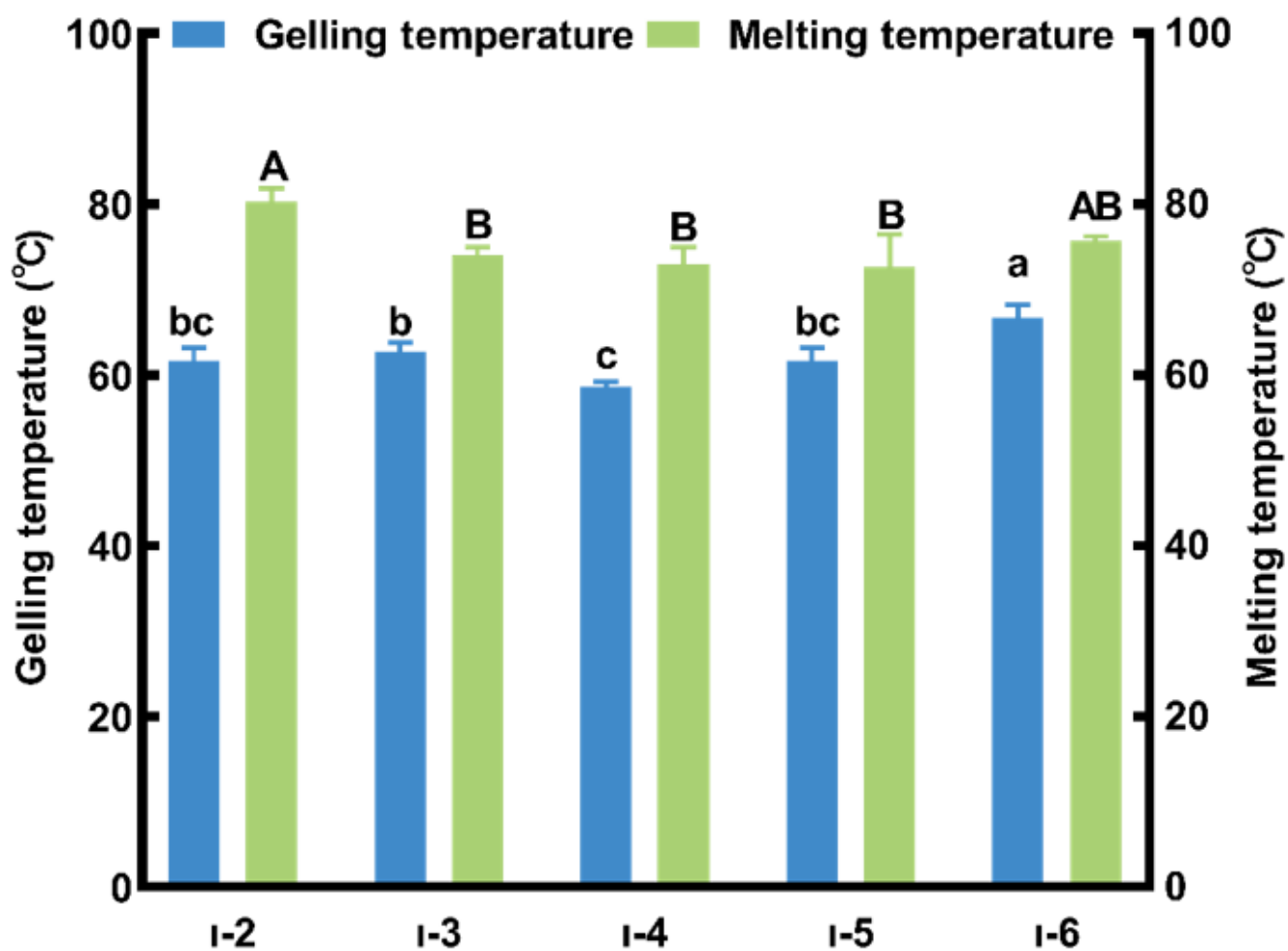


Figure 3

The melting and gelling temperature of ι-carrageenan

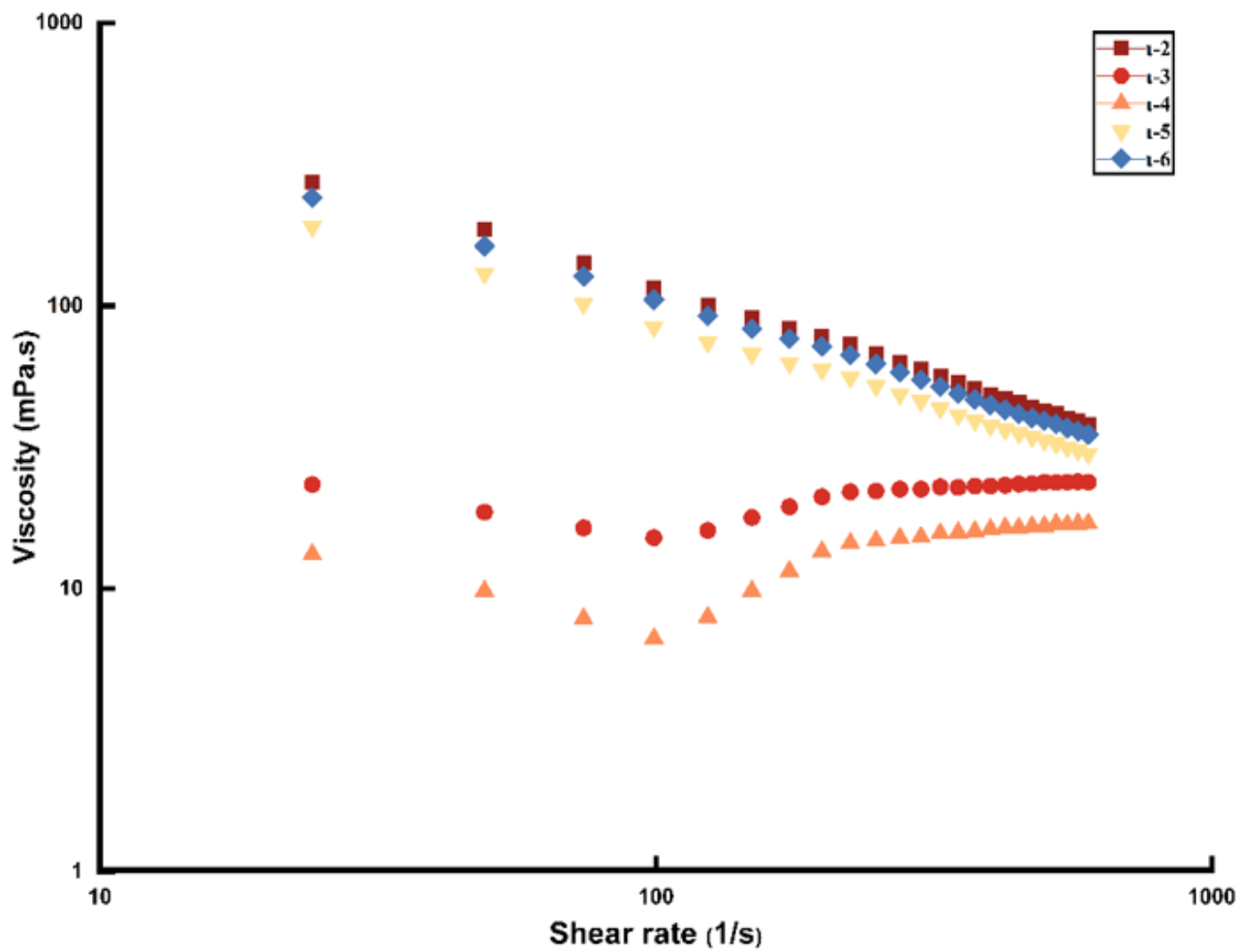


Figure 4

The apparent viscosity of ι-carrageenan

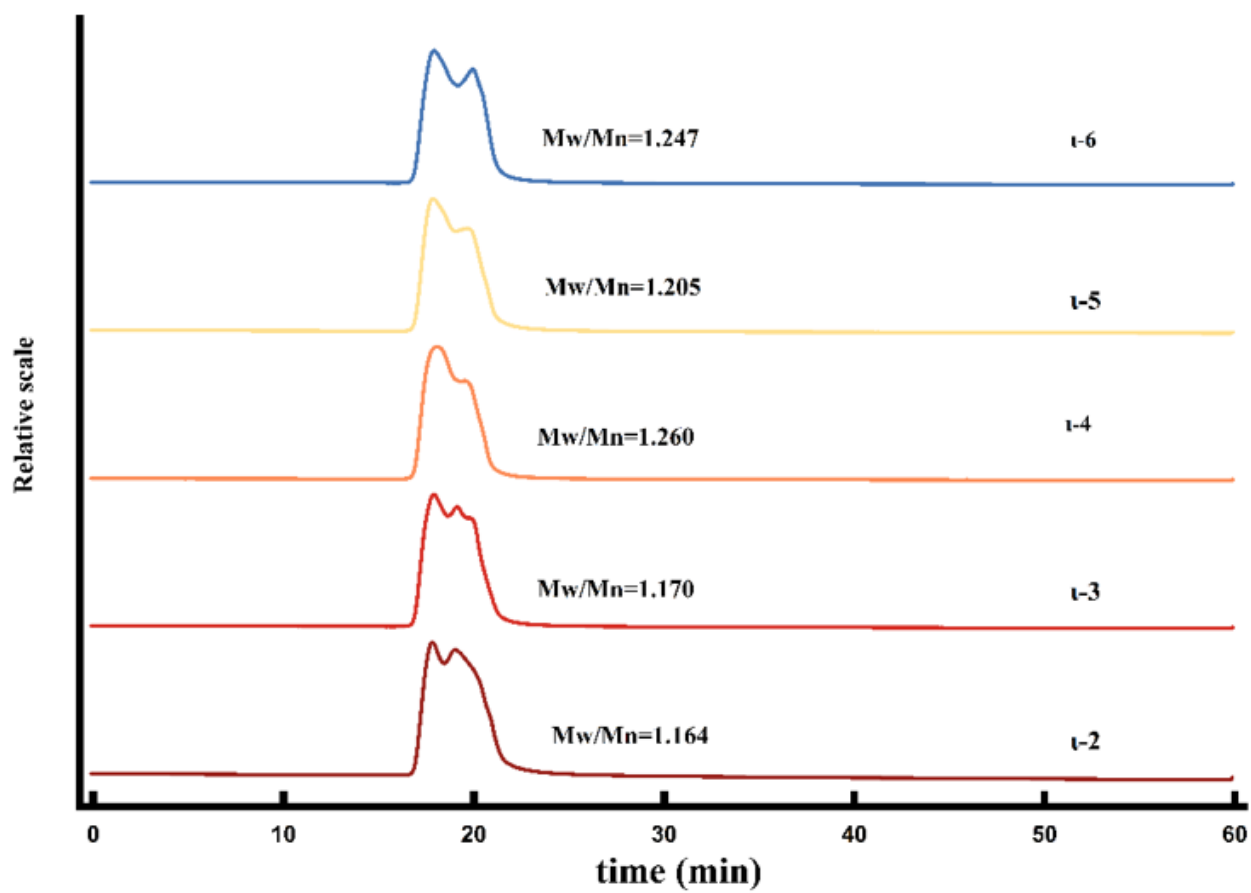


Figure 5

The molecular weight of ι-carrageenan

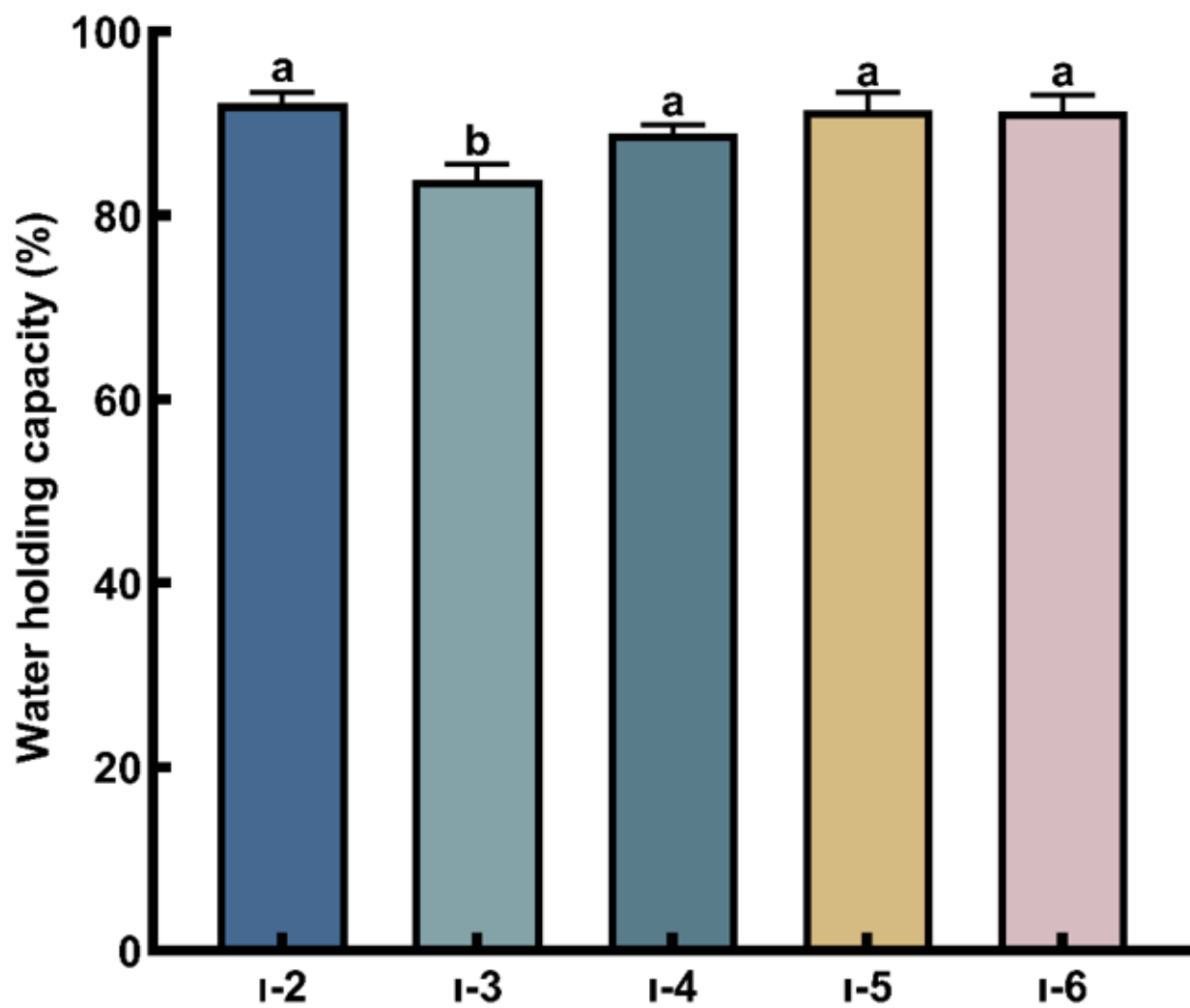


Figure 6

The WHC of ι-carrageenan

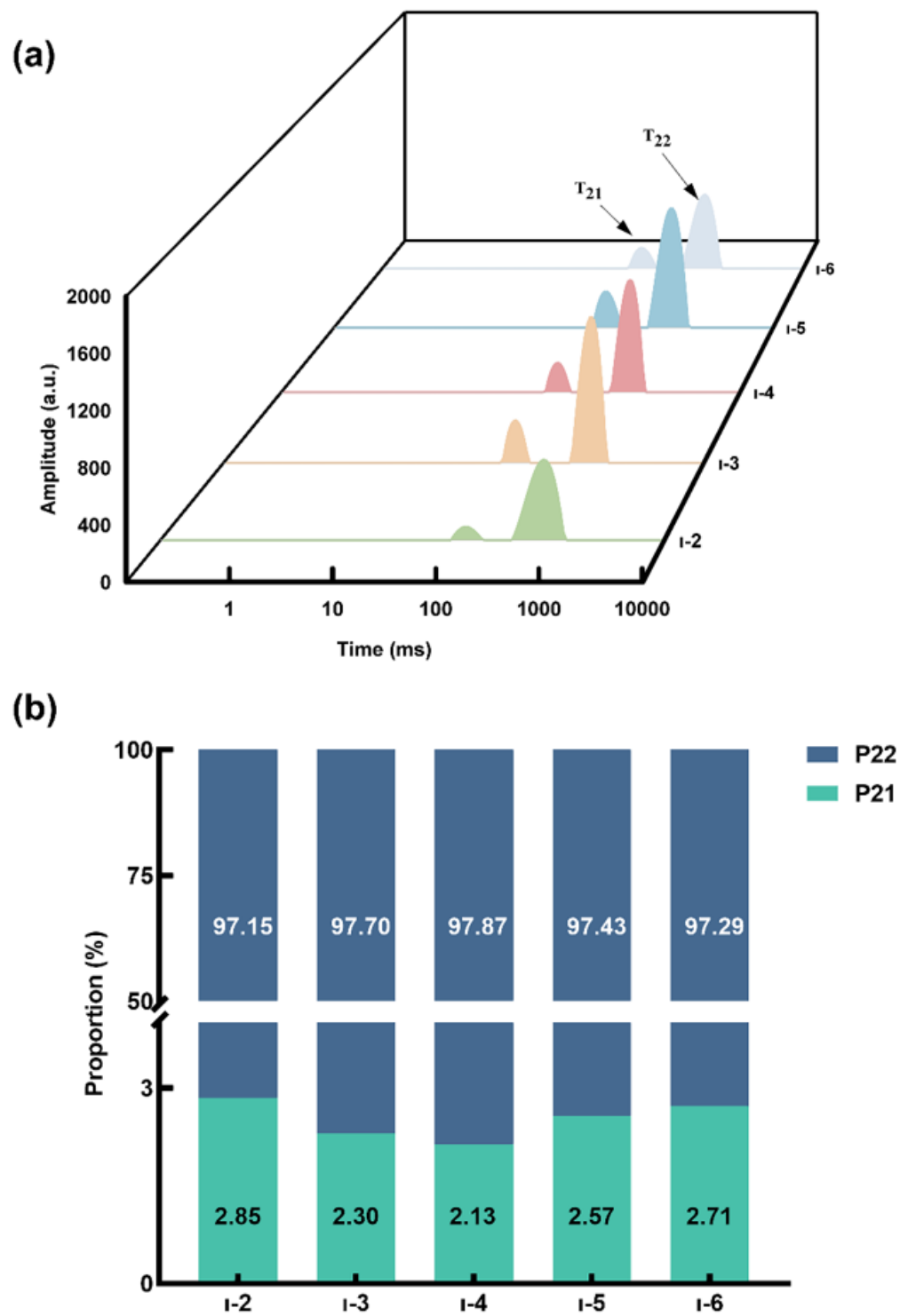


Figure 7

The moisture distribution of carrageenan

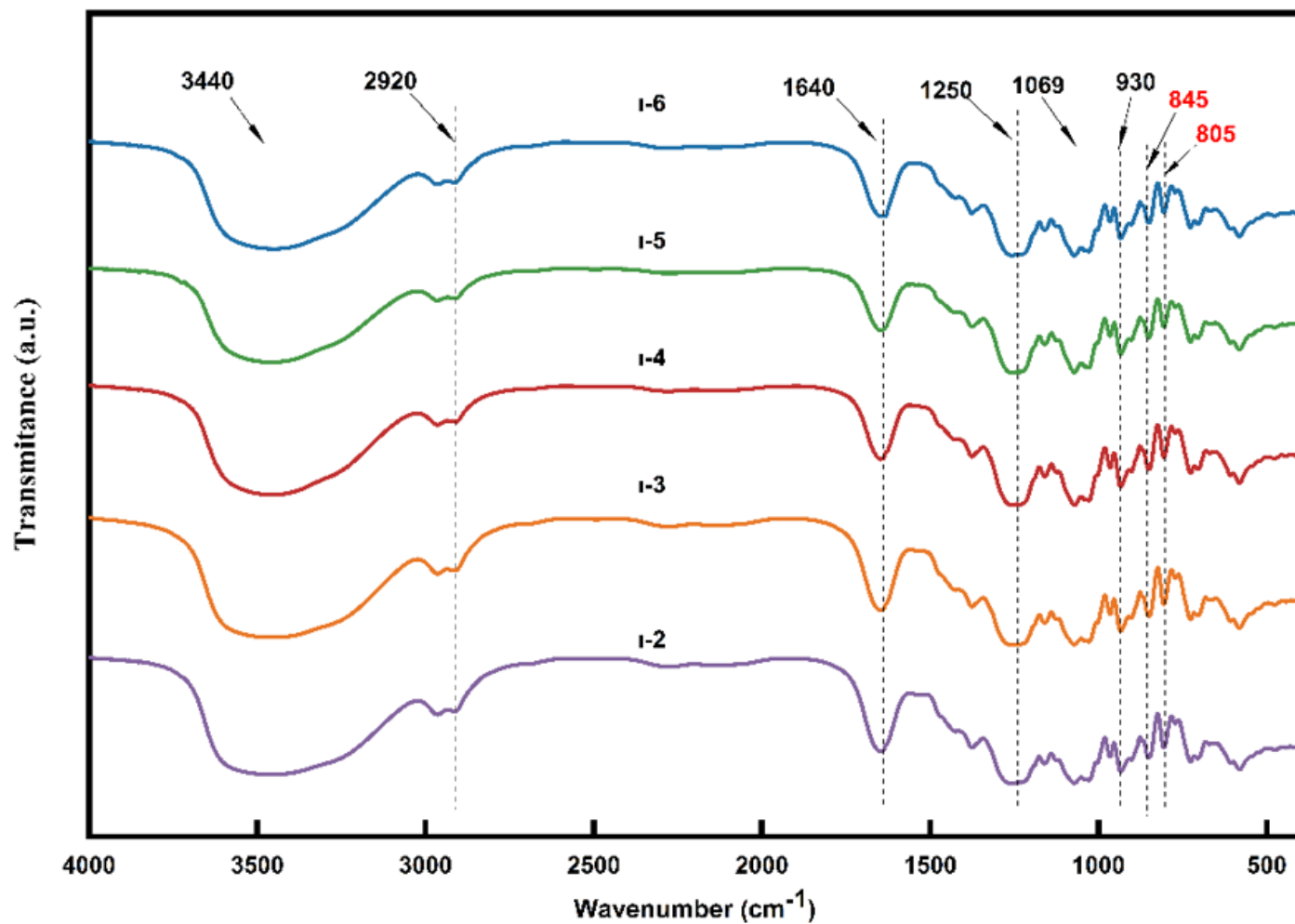


Figure 8

FT-IR spectra of ι-carrageenan

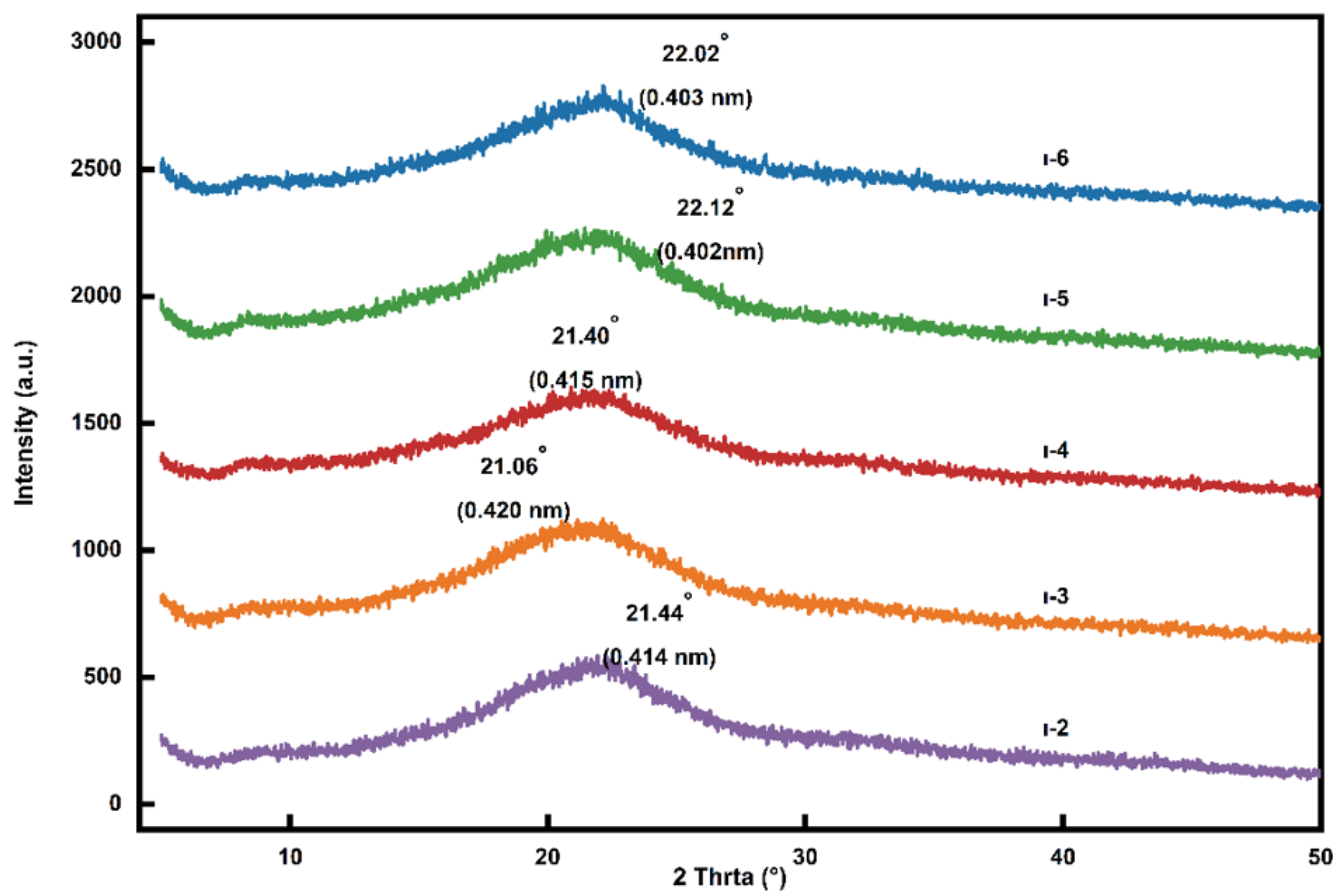


Figure 9

XRD spectra of ι-carrageenan

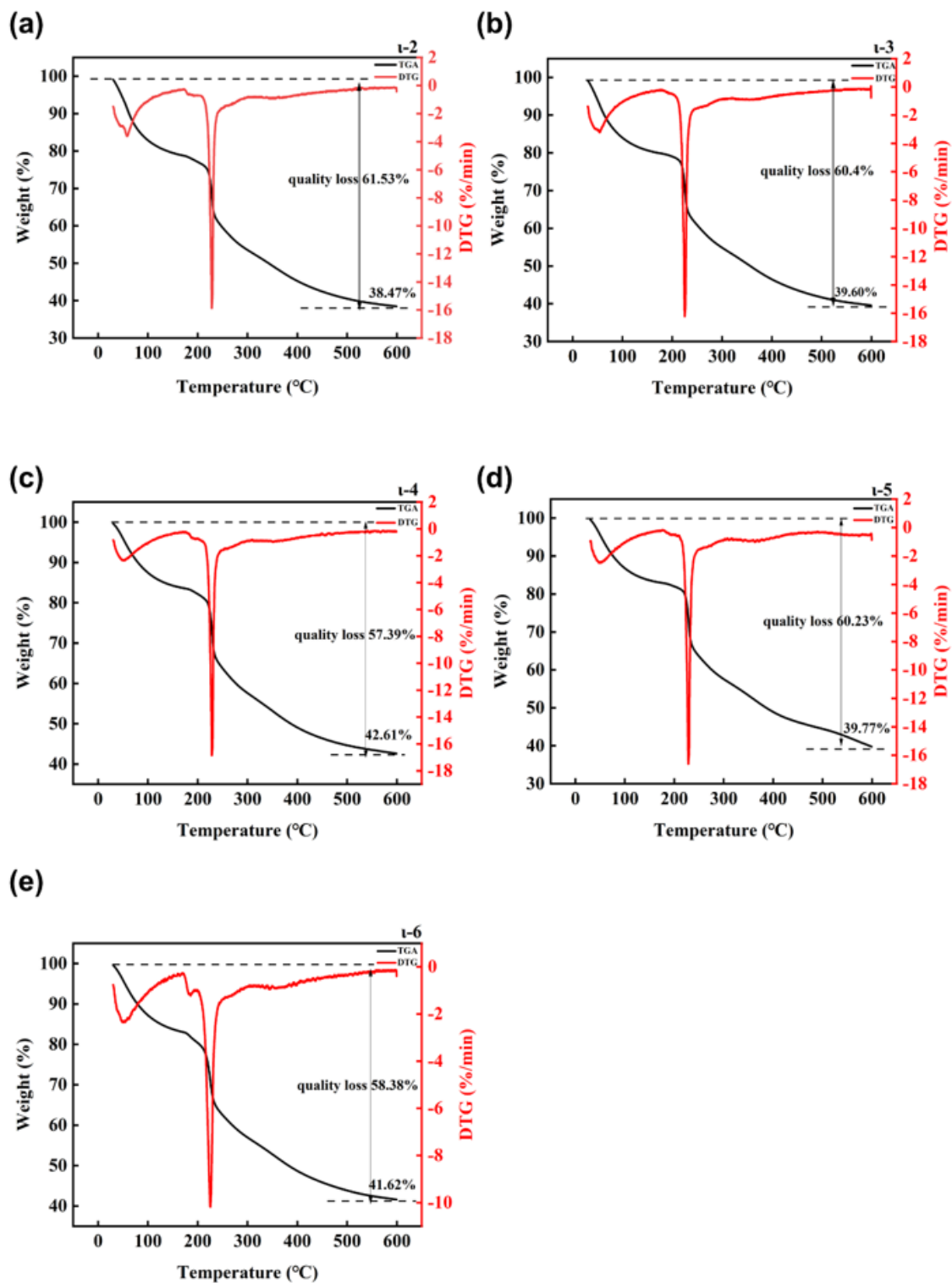


Figure 10

TGA analysis of ι -carrageenan (a) ι -2, (b) ι -3, (c) ι -4, (d) ι -5, (e) ι -6

Supplementary Files

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