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Research Article

The Effect of Extraction Conditions on Chemical and Thermal Characteristics of Kappa-Carrageenan Extracted from *Hypnea bryoides*

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Hypnea bryoides collected from the Arabian Sea on the southern coast of Oman was investigated for κ-carrageenan optimal extraction conditions. The effects of different conditions of alkali treatment (4, 6, and 8% w/v NaOH), temperatures (70, 75, and 80°C), and time (2, 2.75, 3.5 hours) on carrageenan yield and chemical and thermal properties were evaluated. Yield was significantly affected by alkaline concentration and temperature, with highest value of 26.74 ± 5.01%. Molecular weights of the extracted carrageenan were significantly reduced by increased temperatures and ranged from $5.95 \pm 0.49 \times 10^5$ Da to $13.90 \pm 0.14 \times 10^5$. FTIR showed that samples under all extraction conditions were similar and confirmed the presence of κ-carrageenan with no traces of μ-precursor. Sulfate content was also significantly reduced by alkaline concentration (from 4% to 6%) and ranged from 7.62 ± 5.52% to 17.02 ± 0.14. Thermal properties showed more sensitivity towards temperature and alkaline strength parameter than time. In addition, melting and gelling temperatures were significantly correlated with the molecular weight, but not sulfate content. In conclusion, mild extraction conditions were found to be more efficient in introducing the intended structural modification while getting the highest yield and quality.

1. Introduction

Among marine macroorganisms, seaweed gained considerable significance due to its utilizations as a rich raw material for extraction of various valuable materials, for instance, polyphenols, organic pigments, proteins, unsaturated fatty acids, and polysaccharides [1, 2]. Commercially, the most significant seaweed polysaccharide is carrageenan followed by agar and alginate. The global market value of carrageenan has improved from US$ 527 million in 2009 to US$ 626 million in the year 2015 [3].

Carrageenans, which are a family of linear sulfated polysaccharides, are found and extracted from the cell wall of certain species of red seaweeds (Rhodophyta) [4]. These natural polymers are capable of forming thermoreversible gels or viscous solutions when added in small concentration to salt solutions. Therefore, they are commonly used as texturing, thickening, suspending, or stabilizing agents in a variety of industrial applications ranging from food products, pharmaceutics, and cosmetics to experimental medicine [5–7].

Rhodophyta of different species, such as *Gigartina, Chondrus crispus, Eucheuma, and Hypnea,* have been revealed to be good source of carrageenans [8] and based on the source, different types of carrageenans can be acquired. From the view of chemical structure, carrageenans are consisted of alternating (1⟶3)-linked β-D-galactose and (1⟶4)-linked α-D-galactose, where (1⟶3)-linked β-D-galactoses are incompletely sulfated at specific positions of C2 and/or C4, C3, and C6; and the (1⟶4)-linked α-D-galactose units are infrequently 3,6-anhydrated (3,6AG) [9]. The differences in the content and position of sulfate group in galactose units resulted in different carrageenan structures, which as a result differ in the rheological features of solutions

and gels. To date, three types of carrageenans have been reported to have commercial significance: kappa (κ), iota (ι), and lambda (λ) [5]. The major variation among kappa, iota, and lambda carrageenan at the molecular level is the amount along with position of the sulfate ester groups which produces different properties [10]. λ-carrageenan contains no hydrophobic 3,6AG bridge but has three hydrophilic sulfate ester groups, which makes this carrageenan readily water soluble under most conditions. κ-carrageenan possesses a 3,6AG bridge and no more than one sulfate ester group, making this carrageenan less hydrophilic and less soluble in water. ι-carrageenan is in-between, with a 3,6AG bridge and two sulfate ester groups. Accordingly, characterization of carrageenan is the most crucial for quality control and to build up new application supported by their unique intrinsic properties [11].

The yield and quality of the extracted carrageenan confirm the commercial significance of both carrageenan and the mother plant to the industry. However, both yield and quality are very much influenced by several factors such as environmental (salinity, pH, temperatures, light intensity, and water movement), physiological (nutrient uptake, growth rate, tissue age, stress tolerances, and defences), and extraction conditions [11–16]. The major common and essential step in carrageenan extraction procedures is alkali treatment at raised temperature for specific time. This step is crucial to verify transformation of the biological precursors to the commercial grade carrageenan, (μ- and ν-carrageenan into κ- and ι-carrageenan, respectively) [11]. The treatment with alkali induces desulfation of the polysaccharide, causing formation of a 3,6AG bridge (between C3 and C6 in the 4-linked-α-L-galactose units) which leads to increase in the gel strength [17]. The increase in gel strength is attributed to the fact that presence of sulfate at C6 of the α-L-galactose residues in the precursor units acts as a "Kink" to prevent the double helix from forming during gel formation. Closure of the ring to form the 3,6AG and elimination of the C-6 sulfate group makes the chain straighten and leads to great regularity in the polymer, resulting in enhancing gel strength due to increased capability of forming a double helix [17]. The severity of alkaline treatment (concentration and temperature) as well as duration affects the yield and quality [18] as inadequate treatment will not cause the required transformation. Moreover, existence of substantial amounts of the natural precursor units in commercial carrageenan preparations has a critical negative effect on the functional (e.g., gelling) properties [19].

Although the general steps in the extraction process are known, the extraction variables do differ [20] as seaweeds differ in their composition and conditions and stage of growth [12–16]. Therefore, alkali treatment of each algal species must be developed and variables like temperature, alkali concentration, and extraction time must be optimized to induce as much desulfation as possible, while still avoiding the yield losses due to degradation and leaching caused by the treatment [21]. Such loss is frequently associated with elevated extraction temperatures and prolonged extraction time [22–24]. Furthermore, industrial production of alkaline-treated-carrageenan produces considerable pollution in the outflows which then must be neutralized, thereby raising production costs [18].

Among the recognized seaweeds in Omani coasts, *Hypnea bryoides* was found to have potential to be a good source for carrageenan. Al-Alawi et al. [25] found that *Hypnea bryoides* gives yield of about 30-32% (d.b.) carrageenan under alkaline condition extraction (6% w/v NaOH, 80-85°C, 4 h). Although the gel gave good property when it was compared with a reference carrageenan, purity test showed that the yield contained only 32-36% carrageenan and 55-57% was salt. No other conditions were tested to see the effect of different conditions on yield and quality. Therefore, the aim of this study was to investigate the effect of various conditions (alkali strength, temperature, and duration time) on yield and characteristics (chemical and thermal characteristics) of κ-carrageenan obtained from *Hypnea bryoides*. As there is little available information on this seaweed in the literature, this study will help in determining the best possible quality of carrageenan from *Hypnea bryoides* which will influence any future marketing plans.

2. Materials and Methods

Standards, chemicals, and solvents used in this study were obtained from Sigma-Aldrich Co. Ltd., and all were of analytical grade unless otherwise specified. All solutions were prepared with deionized water. All samples were analyzed at least twice.

2.1. Sample Collection. Based on our previous work [25], samples of the red seaweed species *Hypnea bryoides* were harvested by local specialists from the southern coast of Oman from Mirbat city (16°59′28.7″N, 54°41′27.7″E) in November 2015. The samples were transferred to the laboratory at Sultan Qaboos University in cold boxes via air, washed with running fresh water, cleaned by hands from foreign matter upon arrival, and then sun-dried under the shade for three days. The dried samples were then packed in plastic bags and stored in a refrigerator at 4°C until further analysis.

2.2. Extraction Method. The alkaline treatment extraction was performed according to the method described by Al-Nahdi et al. [26]. The method involved rehydrating twenty grams of the sun-dried samples overnight in 1L of deionized water and then depigmentation in 100 ml of acetone/methanol (1:1) mixture. The samples were then treated with alkaline solution (1.5L) of variable strengths (4, 6, and 8% w/v of aqueous NaOH solution) and heated at different temperatures (70, 75, and 80°C) for different intervals (2, 2.75, and 3.5 hours). Occasional and gentle stirring was employed after the first hour of heating. Then, the algal insoluble materials were collected from the solution using double layers of cheesecloth (coarse filtration) and washed three times with deionized water. The retained algal materials were again redissolved in 500 ml of deionized water, pH adjusted to 7 using 6N HCl, and then heated to 90°C for 1 h with continuous gentle stirring. The insoluble algal materials were separated and discarded from the hot mixture using

double layers of cheesecloth and Whatman GF/D, GF/C filter paper. Then, the pH of the collected liquid was checked and adjusted to 7 and kept overnight (14h) at 4°C in a refrigerator to form gel. Volume of the gel was reduced to one-half by evaporation in an air forced oven at 60°C for 12 h. The concentrate was dialyzed against deionized water for 72 h using dialysis membrane with 12000-14000 Daltons cut-off (Medicell International Ltd, London) and dried afterwards for 18 h at 40°C in vacuum oven.

Finally, the dried extracts were milled and then stored in a sealed plastic container at room temperature until further analysis. Two replicates were conducted for each combination of alkali concentration, temperature, and treatment duration.

2.3. FTIR (Fourier Transform Infrared) Analysis. The FTIR analysis was performed according to the method described by Al-Alawi et al. [25] using Magna 560 FTIR spectrometer (Thermo Nicolet, USA) equipped with ZeSn ATR cell and DTGS detector. The IR spectrum was collected by averaging 128 scans at resolutions of 4 cm^{-1}.

2.4. Sulfate Content Determination. Sulfate content was determined using the method described by Dodgson [27]. In brief the method involved mixing 1% w/v carrageenan solution (0.2 ml) with 4% (w/v) trichloroacetic acid (3.8 ml) and 1 ml of the $BaCl_2$-gelatin reagent. The mixture was allowed to stand for 15 min at room temperature for color development and then absorbance was recorded at 360 nm. A calibration curve was prepared with solutions of K_2SO_4 containing 20-200 μg of SO_4^{2-} ions.

2.5. Molecular Weight Analysis. Molecular weight determination experiment was conducted as described by Al-Nahdi et al. [26]. The analysis was carried out on Agilent 1100 (Agilent, USA) instrument equipped with differential refractive index detector (Agilent RID 1100). The separation was achieved using Waters Ultrahydrogel Linear column (Waters Corporation) and the mobile phase 0.1M NaCl solution with isocratic elution. A sample size of 20 μl of 0.1% w/v of carrageenan solution was injected in the system at a flow rate of 1 ml/min. The temperature of the column compartment and the detector flow cell were maintained constant at 40°C.

2.6. Differential Scanning Calorimeter Measurements. The procedures for Differential Scanning Calorimetry measurements (DSC) were similar to those used previously by Al-Alawi et al. [25] with minor modifications. The thermal analysis measurements were carried out using a modulated differential scanning calorimeter (model Q1000, TA Instruments, USA). The tested gels were made up of 1.5% (w/v) of the extracted carrageenan and ionic strength of 30 mM KCl in an aqueous environment. The samples were scanned during a heating/cooling cycle (25-90-25°C) at a rate of 5°C/min, with an empty pan as a reference.

2.7. Statistical Analysis. The statistical analysis work was performed with the SPSS software version 11.5. The obtained data were presented as mean ± standard deviation. General Linear Model (GLM) for univariate analysis was employed to determine the main and interactive effects of alkali treatment conditions on carrageenan yield, molecular weight, and sulfate content. When significant differences were found, a multiple post hoc Duncan's Multiple Range Test was applied. P values<0.05 were considered as statistically significant.

3. Results and Discussion

The yield and chemical properties of alkali-treated carrageenan extracted from *Hypnea bryoides* are summarized in Table 1.

The results demonstrate that the total yield value obtained from different alkali treatments extraction conditions ranged between 6.59 ± 0.09% and 26.74 ± 5.01%, obtained by the treatment with 8% NaOH, 3.5h, 80°C and 6% NaOH, 2.75h, 70°C, respectively.

In general, some of the carrageenan yields obtained in this study were in the same range of those extracted in aqueous media obtained by Yermak et al. [29] from *C. pinnulatus* (20.5%-18.2), Reis et al. [30] from *H. musciformis* (48%-21%), and Webber et al. [31] from *K. alvarezii* (35.8%-18%). However, all the abovementioned studies were performed without alkaline treatment step and also without dialysis step, which raise questions on purity and quality of the reported yield. Therefore, the comparison based on the absolute yield is misleading.

For the alkaline treated carrageenan, yields found in this study were in the range of those reported for other species such as 26.2% from *H. porphyroides* (Indian Ocean) [32], 29.1% from *H. durvillei* (Philippines) [33], 20–32% from *K. alvarezii* (Brazil) [34, 35], 15% from *H. durvillei* (Madagascar) [36], 26.8% from *H. floresii* (México) [37], and 25.8-37.2% from *E. spinosum* (Indonesia) [38].

On the other hand, the values from the present study are slightly lower compared to results obtained from other species such as *G. skottsbergii, S. crispata, and C. crispus, G. atropurpurea* (31-44%) [39], *E. isiforme* (43.5%, 33.8%) [14], and *K. alvarezii* (Doty) (30% to 39%) [28].

The differences in yield between different species is very much understood due to the morphology differences as explained above; moreover, difference in the same species grown in different geographical area has been also noticed and it was attributed to differences in the harvest time, growth conditions (salinity, deepness, and nutrients), time of growth, environmental conditions (wind speed, precipitation, cloud cover, insulation, water and air temperature, and length and period of waves), and extraction process and parameters as reported by several studies [11–16, 32].

In this study, the yield that was obtained by the condition (6% NaOH, 3.5h, and 80°C) is very low (12.69%) compared with the previously reported yield (33.2%) for the same plant collected from the same geographical area and extracted under nearly the same conditions (6% NaOH, 4 h, and 80-85°C) [25]. The reason lays behind the fact that in the previous study no dialysis step was performed as the case in the current study. After correcting for the dialysis step in the previous study (subtracting the salt from the yield), the yield

Table 1: Properties of Carrageenan extracted from *Hypnea bryoides* under different alkali pre-treatment conditions.

Treatment			Yield	M_W	Sulfate
NaOH (w/v)	Time (h)	Temp. (°C)	(% w/w)[1] ± SD	(Dalton) ± SD	(% w/w)[1] ± SD
4%	2	70	21.72. ± 3.61	$(13.15 \pm 0.64) \times 10^5$	07.62 ± 5.52
		75	23.44 ± 0.01	$(11.50 \pm 1.27) \times 10^5$	17.42 ± 2.97
		80	14.86 ± 3.72	$(07.00 \pm 7.20) \times 10^5$	19.22 ± 0.14
	2.75	70	17.62 ± 3.17	$(12.85 \pm 1.30) \times 10^5$	15.12 ± 3.68
		75	18.95 ± 2.59	$(11.30 \pm 0.57) \times 10^5$	13.82 ± 9.19
		80	13.24 ± 3.85	$(07.50 \pm 6.51) \times 10^5$	20.52 ± 0.00
	3.5	70	21.90 ± 0.05	$(13.10 \pm 0.14) \times 10^5$	15.92 ± 0.00
		75	15.12 ± 2.60	$(12.20 \pm 0.00) \times 10^5$	18.82 ± 3.82
		80	09.37 ± 2.37	$(12.40 \pm 0.00) \times 10^5$	21.22 ± 1.27
6%	2	70	23.06 ± 5.06	$(11.80 \pm 0.00) \times 10^5$	11.82 ± 4.38
		75	11.87 ± 1.53	$(11.70 \pm 0.71) \times 10^5$	16.42 ± 0.99
		80	15.21 ± 3.62	$(11.30 \pm 0.00) \times 10^5$	15.82 ± 3.25
	2.75	70	16.95 ± 7.39	$(10.20 \pm 0.14) \times 10^5$	14.02 ± 7.78
		75	10.15 ± 3.73	$(10.00 \pm 1.84) \times 10^5$	16.72 ± 0.28
		80	12.16 ± 3.99	$(06.65 \pm 5.44) \times 10^5$	09.82 ± 2.40
	3.5	70	26.74 ± 5.01	$(13.90 \pm 0.14) \times 10^5$	17.02 ± 0.14
		75	11.06 ± 2.62	$(10.90 \pm 1.41) \times 10^5$	16.52 ± 2.26
		80	12.69 ± 6.37	$(06.10 \pm 6.93) \times 10^5$	15.42 ± 2.69
8%	2	70	11.58 ± 1.79	$(12.30 \pm 1.13) \times 10^5$	19.62 ± 0.99
		75	11.30 ± 0.74	$(12.35 \pm 0.49) \times 10^5$	25.52 ± 1.70
		80	10.24 ± 1.07	$(07.25 \pm 6.15) \times 10^5$	21.52 ± 0.00
	2.75	70	10.85 ± 1.97	$(07.10 \pm 7.07) \times 10^5$	20.92 ± 1.98
		75	11.06 ± 0.96	$(05.95 \pm 0.49) \times 10^5$	25.32 ± 2.26
		80	08.22 ± 0.34	$(07.90 \pm 5.23) \times 10^5$	24.72 ± 1.41
	3.5	70	17.38 ± 2.39	$(08.05 \pm 7.14) \times 10^5$	23.92 ± 4.81
		75	07.58 ± 0.77	$(11.25 \pm 0.21) \times 10^5$	28.12 ± 0.57
		80	06.59 ± 0.09	$(06.75 \pm 6.58) \times 10^5$	21.72 ± 0.85

[1] Dry basis.

came down to 10% which is close to the yield extracted in this current study under similar conditions and very much lower than the yield (26.74 ± 5.01% or 23.44 ± 0.01%) obtained at the condition of 6% NaOH, 3.5h, 70°C and 4%, 2 h, 75°C, respectively. This finding gives significance to the current study to find the optimum conditions for extraction to maximize the yield with the highest quality. On this regard, it has been found in this study that carrageenan yield significantly reduced with the increase in alkali treatment strength and temperature. Furthermore, statistical analysis showed that the time parameter insignificantly influenced carrageenan yield (Figure 1). The interaction between independent variables was found to be nonsignificant.

This finding was not a surprise, but in contrary it was expected due to partial degradation of polysaccharide chains by alkali moieties, since alkaline extraction operation inevitably involves some degradation of the polysaccharide which accelerates at elevated temperatures [4, 11, 31, 37, 40]. Furthermore, further increase in the alkali concentration could lead to a sharp decrease in yield [4].

The molecular weight (M_W) of the extracted carrageenan is shown in Table 1.

The results demonstrate that the M_W value obtained from different alkali treatments extraction conditions ranged between $(5.95 \pm 0.49) \times 10^5$ and $(13.90 \pm 0.14) \times 10^5$ Da at the conditions 8% NaOH, 2.75h, 75°C and 6% NaOH, 2.75h, 70°C, respectively. The results indicated that the degradation rate was elevated at higher temperatures as lower molecular weight carrageenan was obtained (less than 7.00×10^5 Da at 80°C). The effect of NaOH concentration and time on degradation was noticed in this study, but it was not significant ($p > 0.05$) (Figure 2). Furthermore, the interaction between independent variables was also not significant. Studying the degradation rate during extraction and the possible causes are of utmost importance, since functionality of carrageenans in most food applications depends on molecular weight and is largely lost if it is below 1.00×10^5 Da [41] which was not seen in any of the treatments used. Furthermore, degraded, low molecular carrageenan was reported to cause inflammation in the colon in rodents, which resembles ulcerative colitis,

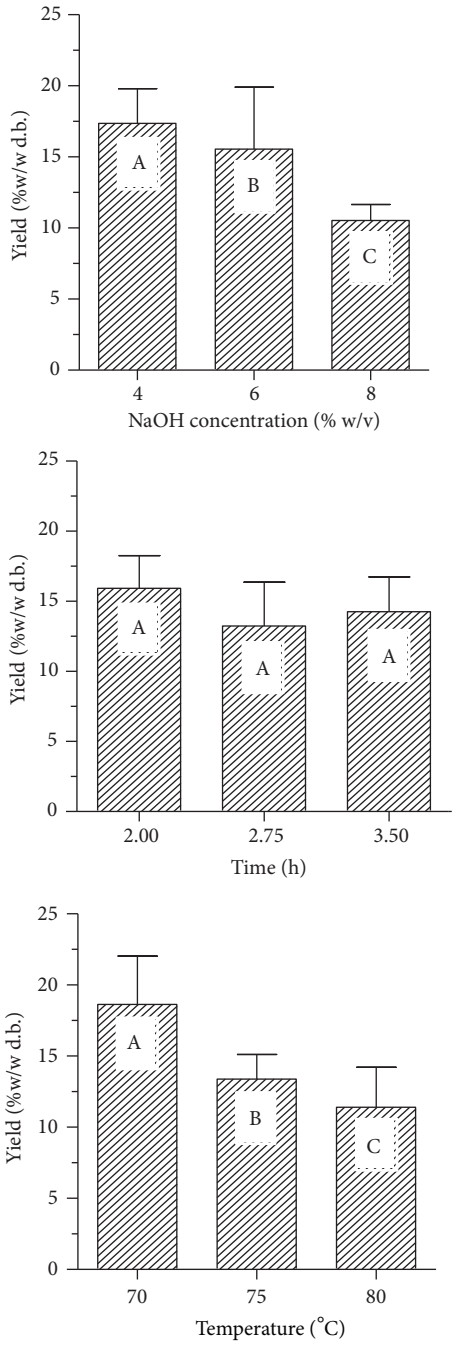

Figure 1: Effects of alkali treatment on yield of carrageenan extracted from *H. bryoides* (means with the same letter are not significantly different ($\alpha = 0.05$)).

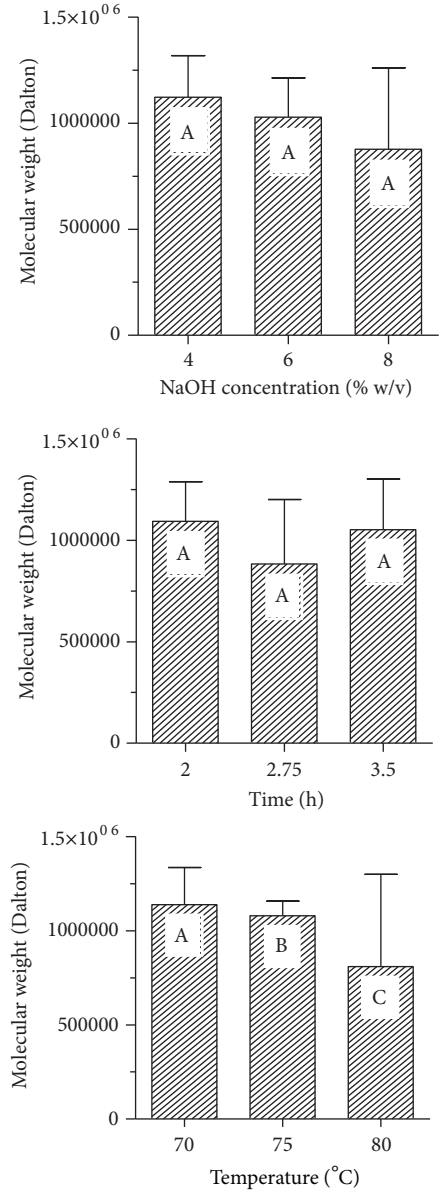

Figure 2: Effects of alkali treatment on M_W of carrageenan extracted from *H. bryoides* (means with the same letter are not significantly different ($\alpha = 0.05$)).

an inflammatory bowel disease [42]. Therefore, low molecular weight carrageenans were classified by the European Scientific Committee [43] and the International Agency for Research on Cancer as a "possible human carcinogen." In this study, any very low molecular weight breakdowns were eliminated through the dialysis step.

Previous study on the same plant [25] gave molecular weight result of 4.1×10^5 Da, which is lower than the current results, probably due to using higher temperatures and longer time compared to this study. Moreover, molecular weights obtained in this study are in the range of results reported by Jupp [44] from *H. bryoides* (6.12×10^5 Da), Hilliou et al. [40] from *Mastocarpus stellatus* (22×10^5 to 4×10^5 Da), and Distantina et al. [11] from *E. cottonii* (10.76×10^5 to 5.48×10^5 Da).

The chemical analyses were further supported by the FT-IR analysis. The collected spectra indicated presence of sulfate esters (S=O) with absorption band in the 1255 cm^{-1} region, 3,6-anhydro-D-galactose at 925 cm^{-1}, and D-galactose-4-sulfate at 845 cm^{-1}. On the other hand, peaks at 830, 820, and 805 cm^{-1}, which are corresponding to D-galactose-2-sulfate (D2S), galactose and D-galactose-6-sulfate (G/D6S), and

Table 2: Peaks assigned to different carrageenan types.

Wavenumber (cm^{-1})	Assignment	Found in Carrageenans*	Extracted Carrageenan from *H. bryoides*
1210-1260	Sulfate ester (O-SO^{-3}) (S)	k, *i*, λ, μ, v, θ, ξ	Present
928-933, 1070 (shoulder)	3,6-anhydro-D-galactose (DA)	k, β	Present
840-850	D-galactose-4-sulfate (G4S)	k, *i*, μ, v	Present
830	D-galactose-2-sulfate (G2S)	λ, θ, ξ	Not found
820, 825 (shoulder)	D-galactose-2,6-disulfate (D2S,6S)	λ, v	Not found
810-820, 867 (shoulder)	D-galactose-6-sulfate (D6S)	μ	Not found
800-805, 905 (shoulder)	3,6-anhydro-D-galactose-2-sulfate (DA2S)	*i*, θ	Not found

*[28].

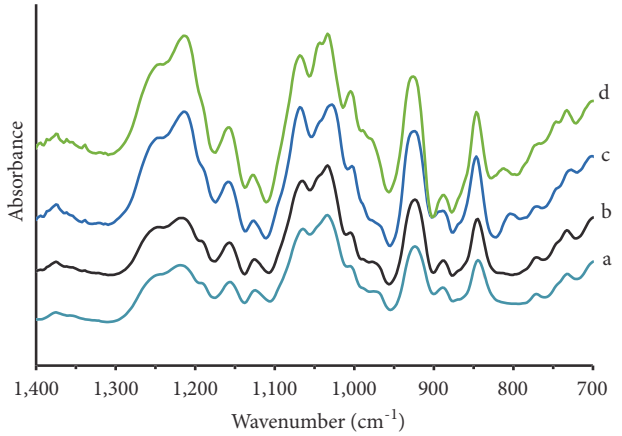

Figure 3: Infrared spectra of carrageenan extracted from *H. bryoides* at 4% NaOH, 2 h, and 70°C condition (a) compared to kappa (b), iota (c), and kappa/lambda (d) commercial carrageenans.

3,6-anhydro- D-galactose-2-sulfate [11, 31, 37] were absent. The 805 cm^{-1} band is characteristic and distinctive of *i*-carrageenan [43] (Figure 3).

The observed absorption bands in all spectra were confirmatory of κ-carrageenan. Table 2 gives the major peaks related to the major functional groups existing on the different types of carrageenans [45]. Results obtained from raw *H. bryoides* plant [25] revealed bands at 872 and 842 cm^{-1} which is assigned to the presence of C-O-SO_4 group on C6 and C-O-SO_4 on C4 of galactose, respectively.

This implies existence of μ-carrageenan in the raw seaweed, which is κ-carrageenan precursor. The nongelling μ-carrageenan is the natural precursor that is present in seaweed that contains κ-carrageenan. The 3,6AG bridges are formed by the removal of sulfate group from the C-6 sulfate ester of the precursor and formation of the 3,6AG bridge [45]. In our case, the lack of 872 cm^{-1} signal band indicates the total conversion of this precursor to kappa form due to the alkaline conditions.

The almost identical FTIR spectra demonstrate that the biopolymers extracted from *H. bryoides* are essentially made of κ-carrageenan (bands at 925 and 845 cm^{-1}) with no μ-precursor. Hence, it is evident from the FTIR spectra that the mildest extraction conditions (at NaOH concentration of 4%, 2 h, and 70°C) were efficient in modifying the biopolymer chemistry and there is no need for further treatments. This result suggests a main advantage of the proposed procedure which will lead to reducing consumption of solvents and extraction time for industry production.

Sulfate is an integral component found in κ-carrageenan used for ionic regulation in the parent plants [46]. Table 1 presents the effect of different treatment extraction conditions on sulfate content. Based on these results, the sulfate percentage of different alkali extraction conditions ranged between 7.62 ± 5.52% and 28.12 ± 0.57% at the conditions of 4% NaOH, 2 h, 70°C and 8% NaOH, 3.5h, 75°C, respectively. The standard specifications for carrageenans sulfate content are in the range of 15-40% [47].

These values of *H. bryoides* are in line with those determined from alkaline treatment analysis available in the literature. Hayashi et al. [34, 35] results ranged from 23.08 to 33.48% for carrageenan extracted from *K. alvarezii* strains from coast of São Paulo state, Brazil. In addition, *E. isiforme* from Nicaragua and *E. isiforme* from Yucatan [14] were between 26.3% and 19.6%, respectively, *E. cottonii* from Indonesia ranged between 11.45 and 16.15% [11], and *H. floresii* from Yucatán Peninsula contained 26.8% [37].

The role of the extraction parameters on sulfate content is illustrated in Figure 4. It is clear that NaOH concentration and temperature have significantly affected sulfate content; in addition, NaOH concentration/temperature interaction (not shown) was also found to have significant effect. With respect to time, there is a noticeable trend where the increasing of time caused the values of sulfate content to increase; however this was not significant.

The results indicated a reduction in sulfate content as NaOH concentration increased from 4% to 6% w/v. From literature, there are many reports on decreasing level of sulfate with increasing concentration of alkaline used for carrageenan extraction [11]. This is due to the reality that formation of 3,6AG bond involves release of sulfate groups; therefore after alkali treatment the sulfate content should be lower. However, the results of the current study do not totally fit in the inverse relationship mentioned above. This is maybe due to the fact that formation of 3,6AG bonds was achieved at the mildest conditions used in this study as it was evident

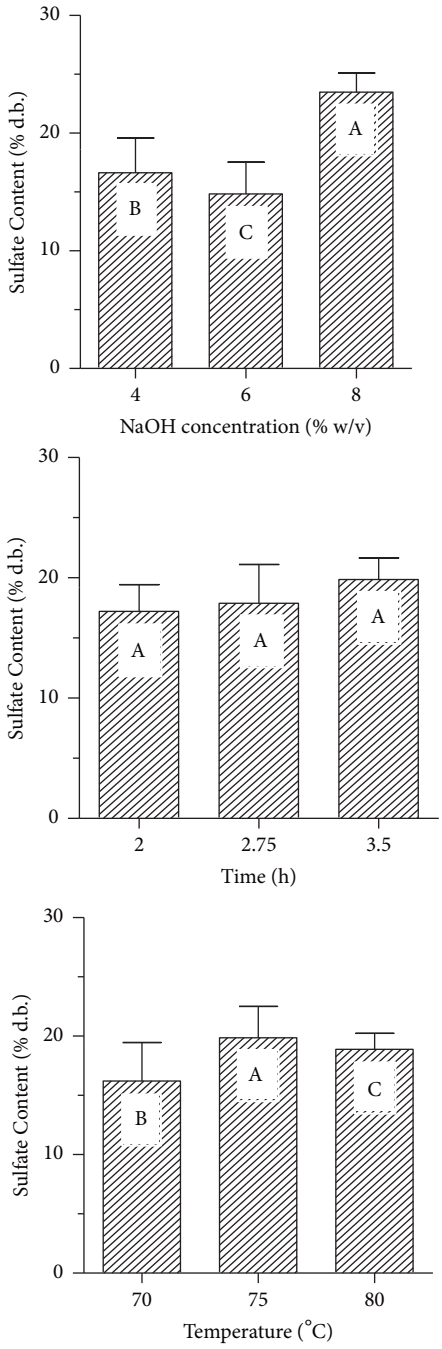

Figure 4: Effects of alkali treatment on sulfate content of carrageenan extracted from *H. bryoides* (means with the same letter are not significantly different ($\alpha = 0.05$)).

from FTIR spectra. Similar findings were reported recently by Moses et al. [48], where the sulfate content increased with the increase in alkaline concentration which was attributed to the removal of protein (due to alkaline hydrolysis) and water soluble low molecular weight compounds which resulted in concentrating the extract. Other authors also obtained different trends than those found earlier. For instance, carrageenan extracted from *H. musciformis* by NaOH (0.1 N) contained sulfate content in the range of 44.1% [49]. On the contrary, alkali treatment (extraction with 0.3 M NaOH, during 4 h, at 90°C) was found to have no effect on the sulfate content of carrageenan extracted from *H. durvillei* [36].

According to the results obtained by DSC measurements, melting and gelling temperatures for the carrageenan gel extracted from *H. bryoides* were in the ranges 47.10 ± 7.01-55.91 ± 2.44°C and 30.25 ± 1.07-35.65 ± 4.73°C (Table 2), respectively. The results of melting temperatures were found to be significantly influenced ($p < 0.05$) by the alkaline concentration (Figure 5(a)) and the interaction of NaOH concentration and time parameters (highest melting temperature was at 4% NaOH concentration and 2.75h), whereas the gelling temperatures were significantly influenced by temperature parameter (Figure 5(b)).

The finding in this study is consolidating what was reported elsewhere [31] where it was reported that gel thermal properties do not seem to be affected much by the extraction time compared to temperature and alkaline strength parameters. Hence extraction time is not the parameter of choice for modifying the end-product chemical structure.

However, the results reported in this study are lower somewhat than those reported elsewhere. For instance, Andrade et al. [50] found that the melting temperatures of gels prepared using κ-carrageenan polymers extracted from *H. musciformis* were in the range 74-75°C, and gelation temperature was around 55°C. Additionally, κ-carrageenan extracted from different strains studied by Sahu et al. [28] had melting temperatures that ranged between 70 and 77°C and gelling temperatures that ranged between 48 and 54°C. Furthermore, the results reported by Al-Alawi et al. [25] on *H. bryoides* κ-carrageenan gel indicated melting at a midpoint temperature of about 70.9°C. The latter example was of the same seaweed species extract, done under equivalent salt concentration and with same concentration of κ-carrageenan solution. However, the extraction parameters were different (6% NaOH, 4 h, 80-85°C) and the sample contained higher concentration of salt (55% of the extract was salt, because no dialysis step was performed). Sen and Erboz [51] mentioned that the addition of salt to κ-carrageenan effectively facilitates the physical gelation. The effectiveness of salts in influencing the phase transition temperatures, gel strength, and the binding state of the strongest junction zones has been shown to play important roles in controlling the viscoelasticity, gelation rate, and syneresis [52]. Furthermore, in the current study, the samples' ion content, as determined by Inductively Coupled Plasma (ICP) Emission Spectroscopy (data not shown), was used to adjust the amount needed for the proper concentration of the polymer. Therefore, comparison between different studies without standardizing all test parameters such as ionic strength is not possible. In the current study no attempts were taken to produce gel with different salt concentrations.

Generally, the alkali pretreatment is performed essentially to improve the gelling properties through lowering sulphate groups in the structure. However, the same treatment may negatively affect the quality features if it is not done properly. This is related to the degradation of the polysaccharide after alkali modification which is corroborated by drastic decrease in molecular weight as it was explained above.

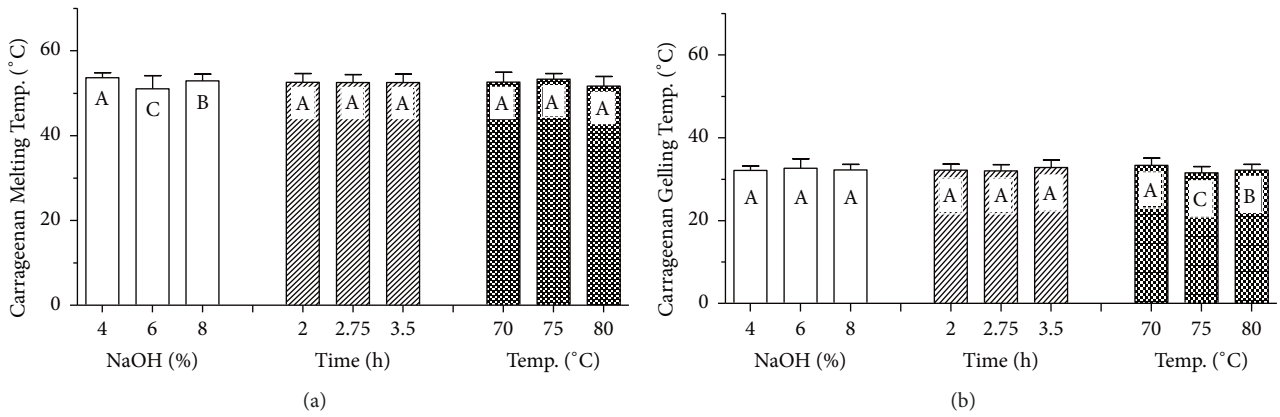

Figure 5: Effect of different parameters of alkaline pretreatment on carrageenan melting (a) and gelling temperatures (b).

The effect of alkaline treatment on the melting and gelation temperatures of the gel prepared from the extracted κ-carrageenan as a factor of molecular weight and sulfate content was tested using Pearson's correlation analysis. A good agreement of significant ($P < 0.05$) direct relationship was found between molecular weight with melting (0.46) and gelling temperatures (0.44). On the other hand, weak insignificant correlation was found between sulfate content and melting (0.05) and gelling temperatures (-0.19).

These results indicate that gel thermal properties do not seem to be affected by the extraction time compared to temperature parameter. Similar results were reported by Webber et al. [31] who concluded that extraction time is not the parameter of choice for tuning the end-product chemical structure of carrageenan and longer extraction times are only preferred to increase the yield in extracted biopolymers structure. Therefore, extraction temperature, or equally pH, is deemed more efficient parameter in modifying the biopolymer chemistry.

4. Conclusion

The current study investigated the effect of different extraction conditions on the different characteristics of κ-carrageenan and the optimal conditions that can be applied for carrageenan extraction from *H. bryoides* grown in Omani coasts to avoid excessive processing (reagents, energy, and time) that would result in degradation of carrageenan molecule and compromise its quality and yield. The recommended conditions based on the above discussion that resulted in a satisfactory yield, M_W, and sulfate content were found to be 6% NaOH for 3.5 hours at 70°C. The FTIR spectra showed presence of κ-carrageenan, with no extent, or minor quantities of ι- or μ-carrageenans in all extracts, which verify the effectiveness of the mild parameters for total conversion achievement.

In addition, κ-carrageenan establishing higher melting and gelling temperatures was satisfied at lower NaOH concentrations (4%) and lower heating temperature (70°C) with the intermediate duration time (2h). Nevertheless, higher κ-carrageenan gels properties observed in the present study were at higher NaOH concentration (8%) which interacted with lower duration times. Interestingly, comparable results can be obtained by lower NaOH concentration (4%). However, in this case, duration times may increase and temperature must be elevated to the maximum. These polysaccharides, on the other hand, would also be of potential utility in the applications demanding low gelation or textural properties, e.g., in personal care or related domains.

Moreover, the present study shows promising commercial potential for *H. bryoides* plant as a source for κ-carrageenan. The results obtained from this study, however, may not be applicable to other *Hypnea* species and other carrageenophytes due to possible variation of seaweed reaction to the treatment. Thus, optimization of the alkali treatment to other carrageenan producing seaweeds should be investigated.

Data Availability

All data used to support the findings of this study are included within the article. Only data of mineral analysis was not provided, but it can be released upon request; however, the authors feel it is not of great importance.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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