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Purification and Characterization of Carrageenan Extracted from Persian Gulf *Laurencia snyderiae* Red Algae

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Abstract

Background and Objective: Carrageenans can be found in a group of red algae called *Carrageenophytes* (*Gigartinaceae*, *Solieriaceae*, *Hypneaceae* and *Furcellariaceae*); however, this substance has not been investigated in *Laurencia* species. In this study, two native species of *Laurencia* within the Persian Gulf were investigated to extract carrageenans. Therefore, the major aims of this study included extraction, optimization and purification of carrageenans from *Laurencia snyderiae*, a native red algae of Persian Gulf.

Material and Methods: *Laurencia snyderiae* and *Laurencia papillosa* were identified based on their morphological characteristics. An experimental design was carried out using Design Expert Software to produce and optimize extraction of semi-refined carrageenans. The software programmed 18 treatments based on temperature, boiling time and KOH concentration. Products of the treatments were prepared for rheometric analyses (viscosity measurements). Optimization was carried out using the software based on the maximum viscosity. Refined carrageenan efficiency was assessed using four extraction methods. Moreover, Fourier-transform infrared spectroscopy and nuclear magnetic resonance spectroscopy spectra were compared. *Laurencia snyderiae* was selected for further investigation.

Results and Conclusion: Based on the rheometric analyses, a semi-refined carrageenan solution was identified as a non-Newtonian pseudo-plastic fluid. The optimum treatment was investigated for *Laurencia snyderiae* at 65 °C for 35 min at KOH concentration of 7% w/v. Results of these two analyses showed that the refined carrageenans from *Laurencia snyderiae* included the lambda type. The highest efficiency was achieved using dialysis method (37%). Based on the abundance of the *Laurencia snyderiae* on the Persian Gulf coasts in all seasons, further studies on carrageenan with higher purities enable use of these substance in various industries. Broader rheological studies can precisely assess characteristics of the investigated carrageenans.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Algae in aquatic ecosystems are the most important primary producers of the food chain [1]. Red algae such as *Kappaphycus* and *Betaphycus* are used to enrich a variety of foods such as chocolates, milks, yogurts and puddings due to their significant quantities of minerals and vitamins. Various species of the Genus *Gigartina*, which includes good

diversity and abundance in marine environments, are used in dairy and carrageenan industries [2]. Red algae are commonly called Rhodophyta due to photosynthetic pigmentation such as phycocyanin and allophycocyanin pigments [3]. The cell wall of red algae includes cellulose and high proportions of mucilage, which is a source of commercial products such as agar and carrageenan [1]. Red algae include several polysaccharides and small quantities of proteins, lipids and minerals; used in polyelectrolytes, pharmaceutical products, nutritional supplements, antimicrobials and polysaccharide products [4]. *Laurencia* spp. are present in warm waters worldwide and often include a significant proportion of tropical and subtropical flora [5]. These species grow in various habitats, including tidal and subtropical zones, rocks and dead rocks and corals [6]. *Snyderiae* is a species of *Laurencia* algae, which includes a dark-brown to purple color. This algal species can be found in all seasons in the lower boundary between tidal surfaces and the surface of rocky subsoil.

In 1862, Stanford invented the term "carrageenin" for the extracted compounds from *Chondrus crispus*. The term became “carrageenan” nearly 25 years ago [7,8]. Carrageenans are derived from a number of Rhodophyceae algae. Carrageenans do not include nutritional values; however, they are used in preparation of foods, pharmaceuticals and medicines for their gelling, thickening and emulsifying characteristics [7,9]. There are several types of carrageenans that are commercially valuable, including kappa, iota and lambda carrageenans [10]. Carrageenans occur in the form of various salts or mixed salts of sulfate ester, including various solubility and gelling characteristics. Kappa and iota carrageenans are dissolved in hot water (over 71 °C). In presence of potassium and calcium cations, they form gelling reagents. Kappa carrageenan gels are fragile and show further leakage, while iota carrageenan gels are more elastic and do not show leakages. The lambda type is soluble in cold and hot water but does not form gels [8]. Carrageenans are sulfated polygalactans that contain 15-40% of sulfate esters with a relative molecular weight of greater than 100 kDa. Higher levels of sulfate esters mean less solubility and lower strength of the gels. Kappa, iota and lambda carrageenans include sulfur ester contents of 25-35, 28-30 and 32-39%, respectively [7,11]. Increases in the gel viscosity is closely linked to its concentration and temperature. Carrageenans can be depolymerized through acid-catalyzed hydrolysis. High temperatures and low pH can rapidly lead to losses of function [7,12].

History of industrial production of carrageenans dates back to the 1940s, when they were developed for use in dairy industries as ideal stabilizers for cacao suspensions in chocolate milks [13]. Carrageenans include anti-HIV effects and their strong anticoagulant activity is used for AIDS therapy. It has been reported that carrageenans include no effects on the binding of viruses or entry of the viral genomes into the host cells but prevent entry of the viral proteins into the cells [12]. Antioxidant activities of the carrageenans have been studied. Lambda carrageenans show the highest antioxidant activity and eliminate free radicals. Research have shown correlations between the sulfate contents and antioxidant activity. Inclusion of carrageenans in diets may lead to decreases in human cholesterol and lipid levels [14]. Other uses of carrageenans include use of them in dairy products, production of dentifrices, preparation of insoluble medicines and industrial suspensions (e.g., watercolor), hardeners and glues in manufacture of textiles and leathers, delivery systems of drugs [12], production of antibiotics [15] and aspartic acid [9], ethanol production from glucose [16], continuous production of acetic acid [17] and industrial wastewater treatment [18]. Extensive uses of carrageenans in various industries have led to increasing uses of this substance globally. In Iran, this substance is not industrially produced. A few and detailed studies have been carried out on carrageenan extraction from native algae of the Persian Gulf. Furthermore, extraction optimization and study of rheological properties of carrageenans have not been carried out in Iran. No reports have been carried out on the extraction of carrageenans from *Laurencia* spp. worldwide. Therefore, the aim of this study was to compare carageenan of *Laurencia* (*L.*) *snyderiae* with those of other species and commercial carrageenans.

2. Materials and Methods

Two samples of red algae were collected from the tidal zones of Bushehr Coast (Persian Gulf), Iran, at low tides. Identification of the samples was carried out based on morphological characteristics using valid key identification [19]. Samples were dried in shade [20]. Lost moisture contents of the collected algae were calculated. Then, samples were washed with tap water for nearly 2 min and dried at 50 °C overnight using oven [20]. Dried algae were powdered using blender [21].

2.1. Initial screening

Initial screening for appropriate algae selection was carried out for further analysis. The two species of *Laurencia* collected from the coastal areas of Bushehr were dried and selected for treatment at KOH concentration of 11% w v^{-1} and boiling temperature of 80 °C (40 min) for each algal species. Rheometric analyses (e.g., tension and elongation) of the products were carried out using Anton Paar MCR301 Rheometer System (Anton Paar, Austria). Then, graphs linked to the rheometric results were produced using Excel 2013 Software. Carrageenans extracted from *L. snyderiae* Dawson showed better rheometric properties. Therefore, the algae were selected for further analyses.

2.2. Optimization with Design Expert Software

Optimization of semi-refined carrageenans was carried out using Design Expert Software. In total, 18 treatments were designed with various KOH concentrations (7-12 % w v^{-1}), boiling temperatures (55-85 °C) and boiling times (110-35 min) using CCD method, including four replications at the central point to assess experimental errors by the software. Treatments were carried out randomly and eventually results were analyzed using Design Expert Software.

2.3. Rheological calculations of semi-refined carrageenans

Rheological calculations were carried out using Anton Paar MCR301 Rheometer (Anton Paar, Austria). Then, semi-refined carrageenan solution (1.5% w v^{-1}) was prepared and incubated at 80 °C for 20 min using water bath [22].

2.4. Extraction of refined carrageenans

There are several methods for the extraction of refined carrageenans. Based on the efficiency of refined carrageenans and available facilities, four methods were selected. It noteworthy that all the four methods for extracting carrageenans of *L. snyderiae* were carried out. After calculation of the efficiency and carrying of Fourier-transform infrared (FTIR) spectroscopy, the best method was selected for nuclear magnetic resonance (NMR) spectroscopy.

2.4.1. Dialysis bag method

The first method included dialysis bag method according to Vairappan et al. [23]. Steps were carried out in the following order of dried algae powder was added to 500 ml of 4% w v^{-1} NaOH solution and 0.25% w v^{-1} $NaBH_4$ and incubated at 80 °C for 3 h at 140 rpm using magnetic stirrer. After cooling down, solution was filtered using filter papers and pH of the solution was adjusted to 7 using acetic acid. Then, neutralized solution was poured into the dialysis bag and set in a sterile distilled water dish on a stirrer for 24 h to complete the dialysis process. Then, solution of the bag was poured into a Petri dish and dried using freeze dryer (CHRIST, Germany) [23].

2.4.2. Calcium hydroxide method

This method is based on Strong's study, which was optimized. Steps of this method were as follows: The shade-dried algae were washed and dried using oven. Briefly, 400 ml of 27% nitric acid solution were poured onto the algae. After 15 min, acid was discarded and distilled water was added to the plate. After 5 min, water was discarded and fresh water was added to the plate. After 5 min, water was discarded. Then, hot water was poured onto the algae, which was transferred on the stirrer for 1.5 h at 80 °C. After 1.5 h, 4 g of calcium hydroxide were added to the plate and the mixture was dissolved on the stirrer. The sample container was incubated at 80 °C overnight. Then, specimen was filterred through filter papers. The pH of the solution was adjusted to 7 using a few drops of acetic acid. Then, isopropanol was added to the solution to precipitate the carrageenans. Supernatant was discarded and the remaining turbid liquid was poured into a Petri dish and incubated at 50 °C to completely dry using oven [24].

2.4.3. Hydrogen peroxide method

This method is based on Gordon and Jonas's study as follows: 250 ml of distilled water were added to the powdered dried algae and mixed well using stirrer. Then, 100 ml of 18% w v^{-1} aqueous calcium hydroxide solution were added to the mixture and incubated at 80 °C for 10 min using stirrer. Then, 2 ml of 3.5% hydrogen peroxide solution were added to the mixture. After 5 min, 100 ml of sodium sulfite solution (8% w v^{-1}) were added and mixed well for 2 h using stirrer. Solution was filterred using filter papers. Then, pH of the solution was adjusted to 6.8-9. Then, isopropanol was added to the solution to precipitate the carrageenans. Supernatant was removed and the remaining turbid liquid poured into a Petri dish and incubated at 50 °C to completely dry using oven [25].

2.4.4. Autoclave method

This method was based on Eswaran et al. Study as follows: 600 ml of saturated calcium hydroxide solution were added to 20 g of the dried algae powder. This was transferred into an autoclave and heated at 121 °C for 15 min at 1.5 bar. Sample was first filterred using tissues and then filter papers. Then, isopropanol was added to the solution to precipitate the carrageenans. Supernatant was removed and the remaining turbid liquid was poured into a Petri dish and incubated at 50 °C to completely dry using oven [26].

2.5. Fourier-transform infrared spectroscopy and nuclear magnetic resonance spectroscopy analyses

The FTIR analysis was carried out to identify the carrageenan type in the refined carrageenan powder. The algal powder was mixed with potassium bromide and transferred onto a measuring plate. The FTIR was carried out using BRUKER VECTOR Manufacturing Machine in Zanjan University Graduate Studies, Zanjan, Iran, at a distance of 500-4000 cm^{-1}. The HNMR spectra were recorded using BRUKER AVANCE 3 400 MHZ (BRUKER, Germany) in the Graduate School of the University of Isfahan, Isfahan, Iran. Analysis was carried out at 55 °C using D_2O solvent. The ^1H spectra were recorded using 32 scans, 5-s relaxation delay and 0.9999-s acquisition time.

2.6. Statistical Analysis

Diagrams were plotted using Excel 2013 Software and ANOVA analysis and equation assessments were carried out using Design Expert Software. Viscosity of the rheometric analysis was recorded by the software and optimized.

3. Results and Discussion

No reports have been available on the extraction of carrageenans from *Laurencia* spp. worldwide. Therefore, this study compared carageenans of this species with those of other associated species.

3.1. Moisture content

The algae were washed with tap water for 2 min according to Normah et al. and gradually dried in shade according to Vairappan et al. [20,23]. Findings by Vairappan et al. have shown that seaweeds dried in shade produce higher carrageenan contents with higher performance and superior characteristics [23]. Then, seaweeds were dried at 50 °C overnight based on Normah and Nazarifah's study [20]. Vairappan et al. have suggested that the acceptable loss of moisture in dry seaweeds, which are appropriate for the carrageenan extraction, is nearly 60-70% of the moisture content [23]. In the current study, quantity of the moisture loss in *L. snyderiae* was 75.26% while the quantity was 78.77% in *L. Papillosa*; hence, the two treatments samples were acceptable.

3.2. Initial screening results

For the primary screening of the selected treatments, rheological properties were investigated for the two samples after preparing semi-refined carrageenan solutions. In non-Newtonian fluids, slope of the shear stress-shear rate diagram (Figure1) is not constant at a given temperature. If the graph collides with the y axis at a point above the origin, it describes a non-Newtonian pseudo-plastic Bingham fluid (27). Figures 2A and 2B show the viscosity-shear rate diagrams. Due to decreases or increases in the viscosity, the fluid is known as shear-thinning or shear-thickening [27]. Figures 2C and 2D show the viscosity-time diagrams. Increases or decreases in viscosity show rheopectic or thixotropic fluids.

Classes of fluids show time-dependent behaviors, meaning that viscosity changes over time even with a constant shear rate. When the viscosity of a fluid (at a constant shear rate) decreases over time, it is called athixotropic fluid [27]. Figure 3 shows the modulus-strain diagram. In this diagram, changes in the elastic modulus (G') demonstrate the critical strain [28]. In Figure 3, the critical strain was 2.19% for *L. snyderiae* (Figure 3A) and 1.48% for *L. papillosa* (Figure 3B), meaning that the fluid behaved like a solid at the strain rates below this critical value and like a fluid above this critical value. Therefore, *L. papillosa* was less resistant against the strain. Marcotte et al. showed that 1% commercial carrageenan solution behaved as a non-Newtonian pseudo-plastic [29]. The two algal species in this study have produced semi-refined carrageenan non-Newtonian Bingham pseudo-plastic fluids. Figures 2A and 2B show that the viscosity was not constant and decreased by increasing the shear stress; thus, fluids derived from the algae were shear-thinning [27]. Figures 2C and 2D show that viscosity decreased over time and subsequently algae produced thixotropic and time-dependent fluids. Garrec et al. verified in a commercial carrageenan study that the carrageenan solution included thixotropic type [30]. Results of the diagrams were quite similar and the only difference was seen in the resistance of the carrageenan solution from *L. papillosa*; hence, *L. snyderiae* was selected to further analysis.

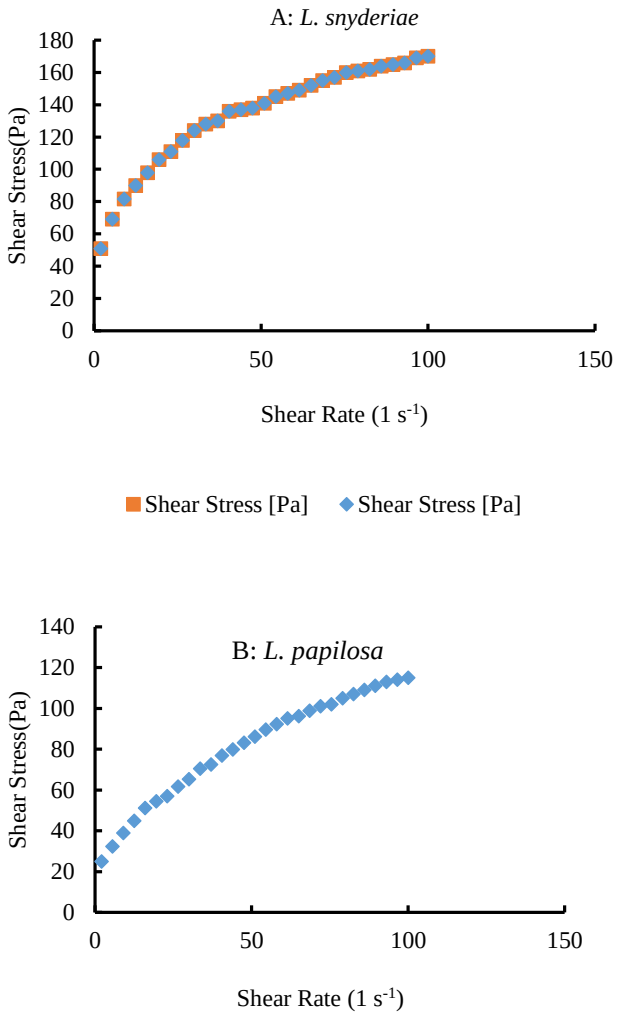

Figure 1. Shear stress-shear rate diagrams. A, *Laurencia snyderiae*; and B, *Laurencia papillosa*

3.3. Design Expert Software

Rheology analyses were carried out using rheometer for all treatments of *L. snyderiae*. Viscosity was selected at the frequency of 25 and data were exported to Design Expert Software (Table 1). Table 2 shows the proposed software grade 3 (Cubic) equation. Figure 4 shows interactions of factors with viscosity. In temperature-KOH graph (Figure 4A), results show that the viscosity decreased at a constant temperature while increasing KOH concentration.

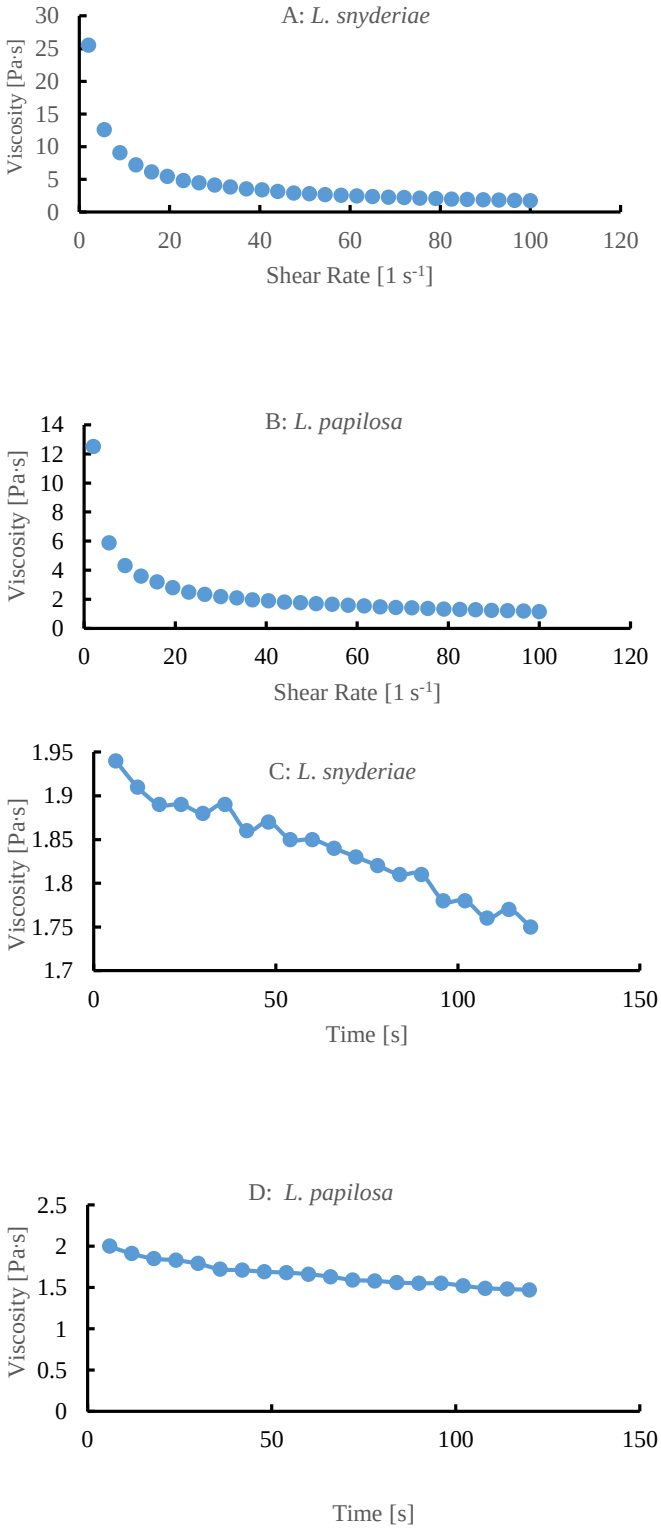

Figure 2. A, Viscosity-shear rate diagram of *Laurencia snyderiae*; B, viscosity-shear rate diagram of *Laurencia papillosa*; C, viscosity-time diagram of *Laurencia snyderiae*; and D, viscosity-time diagram of *Laurencia papillos*

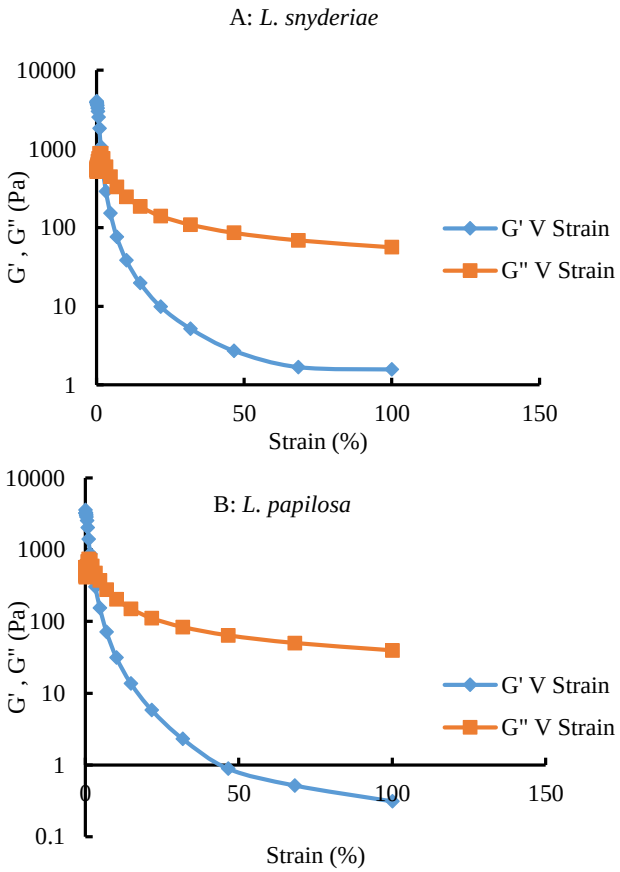

Figure 3. Modulus-strain diagrams. A, *Laurencia snyderiae*; and B, *Laurencia papillosa*

Table 1. Treatment viscosity of *Laurencia snyderiae*

	Factor 1	Factor 2	Factor 3	Response 1
Run	A:KOH W V^{-1}	B:TEMPRATURE C	C:TIME MIN	complex viscosity Pa/s
1	9.5	95.2269	72.5	0.0675
2	7	85	35	0.151
3	7	55	35	10.6
4	9.5	70	135.567	0.117
5	7	55	110	2.73
6	13.7045	70	72.5	0.0953
7	12	55	35	4.15
8	9.5	44.7731	72.5	0.356
9	5.29552	70	72.5	29.4
10	7	85	110	2.18
11	12	85	110	3.02
12	12	55	110	11.4
13	9.5	70	72.5	0.183
14	9.5	70	9.43277	0.105
15	9.5	70	72.5	0.168
16	9.5	70	72.5	0.278
17	12	85	35	2.88
18	9.5	70	72.5	0.201

At a constant KOH, increases in temperature included variable dispersive effects on the viscosity. In time-KOH graph (Figure 4B), the viscosity decreases by increasing the KOH concentration at a constant time. At constant KOH concentrations less than 9.5% with increasing the time, the viscosity decreased. At KOH concentrations greater than 9.5%, increases in time included mild variable effects on the viscosity. In temperature-time graph (Figure 4C) at a constant time less than 72.5 min, increases in temperature decreased the viscosity while this was reversed over 73.7 min. At constant temperatures of less than 70 °C, viscosity decreased as times increased. At temperatures higher than 70 °C, viscosity increased with increasing the time. As previously stated, the highest yield (nearly 37%) was achieved in dialysis method. For optimization, the range of factors was set within the range of the assay and the optimum viscosity range was set at the maximum value for the software. The optimum treatment was reported for *L. snyderiae* at 65.907 °C, 35 min and KOH concentration of 7% w v^{-1} with 15.939 viscosity.

Table 2. Cubic equation of *Laurencia snyderiae*

Final Equation in Terms of Actual Factors:	
complex viscosity	=
-424.82850	
+19.98002	* KOH
+18.86416	× TEMPRATURE
-1.12449	× TIME
-1.72867	× KOH × TEMPRATURE
+0.10807	× KOH × TIME
+0.014983	× TEMPRATURE × TIME
+2.60348	× KOH^2
-0.16001	× $TEMPRATURE^2$
-1.28457E-004	× $TIME^2$
-1.51191E-003	× KOH × TEMPRATURE × TIME
-0.026617	× KOH^2 × TEMPRATURE
+8.10911E-004	× KOH^2 × TIME
+0.016775	× KOH×$TEMPRATURE^2$

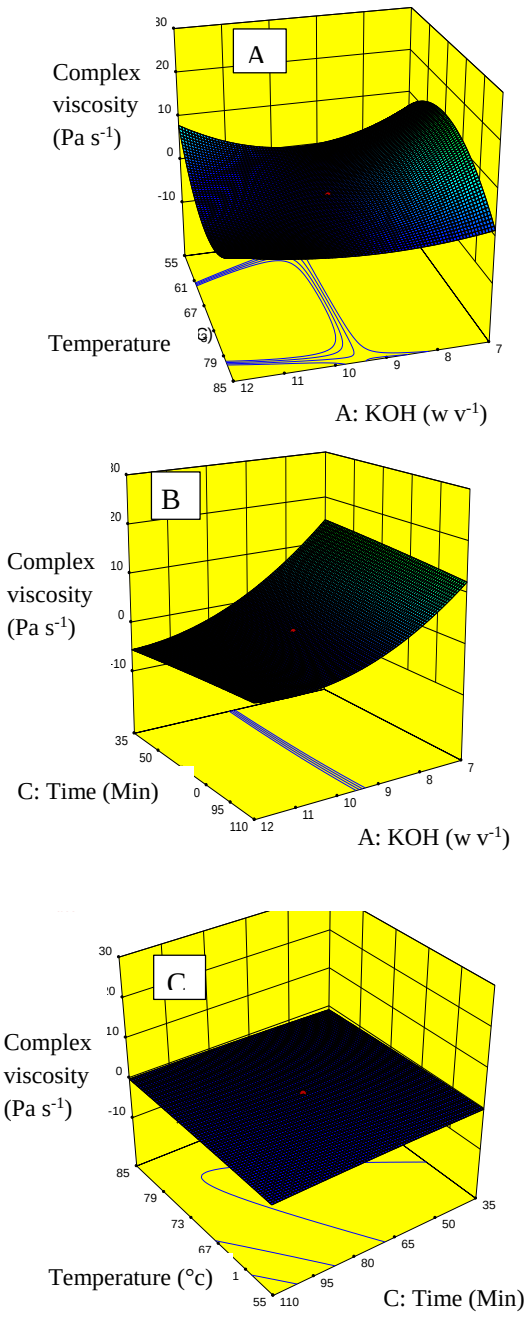

Figure 4. 3D graph of factors interaction on viscosity A. temperature- KOH diagram B. time-KOH graph C. temperature-time graph

3.4. Refined carrageenan efficiency

Yield of the dialysis bag method for *L. snyderiae* was 37%. Changes to the autoclave method (e.g., decreses of duration from 3 h to 15 min) showed a yield of 7.5%. Use of calcium hydroxide method yielded 1.4%. In hydrogen peroxide method, efficiency of *L. snyderiae* was 5.95%. Vairappan et al. used dialysis bag method to extract refined carrageenans from *Kappaphycus alvarezii* Doty algae. The refined carrageenan efficiency was 58.3%. Extraction of carrageenans in this study was carried out according to Vairappan et al. by means of variations including tempe-

rature (80 °C) and filtration (paper-filter) [23]. Hansen et al. extracted all kinds of carrageenans from *Eucheuma spinosum* seaweeds and reported a yield of 49.9% from dry seaweeds. The autoclave method derived from Hansen et al. method [31]. Strong et al. carried out extraction of carrageenans using calcium hydroxide and acid from *Chomlrus crispus* algae. Their results showed that the use of diluted acid helped improve extraction of carrageenans from seaweeds [24]. Gordon et al. extracted carrageenans with a yield of 3.39% using *Chondrus crispus* algae [25]. As previously stated, the highest yield was achieved using dialysis method.

3.5. Fourier-transform infrared spectroscopy and nuclear magnetic resonance spectroscopy analyses

The FTIR analysis was carried out to identify types of the extracted carrageenans. The FTIR spectra were recorded in the range of 400-4000 cm^{-1}. Figure 5A shows FTIR of the dialysis method. The 1021.47 cm^{-1} band corresponded to 1026 cm^{-1} band of the lambda type, previously verified by Webber et al. [32], and 1256 cm^{-1} band near 1220-1260 cm^{-1} range corresponded to the sulfate ester group, verified by Pereira et al. [33] and Volery et al. [34]. In the range of 1010-1080 cm^{-1}, a glycosylated graft band (1049.76 cm^{-1}) was seen, as previously verified by Pereira et al. (33). In the range of 925-935 cm^{-1} (928.69 cm^{-1}), 6-anhydro-D-galactose belonged to lambda carragenens, as verified by Silva et al. [35]. A band was demonstrated at 807.88 cm^{-1}, which could be indicative of the presence of iota carragenens, as verified by Silva et al. [35]. Figure 5B reveals that the FTIR repetition was linked to the method of dialysis; in which, a band was still present at 806.48 cm^{-1} (Iota). In addition, the band at 1021.52 cm^{-1} was linked to the Lambda type, 1051.08 cm^{-1} to the sulfate ester groups and the 2924.47 cm^{-1} band to the C-H content. According to De Velde et al., even commercial samples consisting of a dominant carrageenan are often contaminated with small variable quantities of the two other carrageenans despite various methods of isolation [36]. Figure 5C FTIR refers to the calcium hydroxide method. A band at 1160.3 cm^{-1} was achieved for the band S=O, as previously shown by Pereira et al. [33]. Bands at 872.53, 835.69 and 823.43 cm^{-1} were associated to the Lambda type, as demonstrated by Silva et al. and Webber et al. [32,35]. Figure 5D shows FTIR of the hydrogen peroxide method, which included bands at 2936 cm^{-1} for CH, 1237.16 cm^{-1} band for sulfate groups, 1044.64 cm^{-1} band for glycosylated graft and 874.05 and 833.85 cm^{-1} bands linked to the Lambda type, respectively verified by Pereira et al. [33], and Silva et al. [35] and Webber et al. [32]. Figure 5E shows FTIR of the autoclave method; in which, the dominant band at 2930.62 cm^{-1} was associated to the CH and 874.05 and 872.89 cm^{-1}, respectively verified by Pereira et al. [33], and Silva et al. [35] and Webber et al. [32], refering to the presence of lambda carrageenans.

In comparison of FTIR spectra, hydrogen peroxide method showed better information; therefore, ^{1}H NMR analysis was carried out for the carrageenans from this method. The ^{1}H NMR spectra were recorded for the refined carrageenans extracted at 55 °C using hydrogen peroxide method. The NMR spectra analysis verified results of the FTIR spectra. Based on the previous studies, temperature affects bands of the NMR spectra. In Figure 6, a band at 5.54 ppm was seen that corresponded to lambda carrageenans (α-D-galactose- 2,6-disulfate). The overall ^{1}H NMR spectra were similar to that of Tojo et al. report on lambda carrageenans [37]. Differences between the results of the current study and other studies could be due to the differences in harvest seasons, environmental conditions, types of algae and purities of carrageenans. Therefore, these need further studies under various conditions and seasons with further repetitions.

4. Conclusion

Morphological identification was carried out for two species of *L. snyderiae* and *L. papillosa* collected on the coast of the Persian Gulf in Bushehr. Extraction of semi-refined carrageenans from these two *Laurencia* spp. was investigated. Initial screening of the rheological experiments showed that the carrageenan solution from the two species included non-Newtonian pseudo-plastic behaviors. The *L. papillosa* spp. included the lowest tolerance against stretch and hence was excluded from the study. Further analyses were carried out on *L. snyderiae*. Extraction of semi-refined carrageenans was treated (18 various treatments of various temperatures, boiling times and KOH concentrations) using Design Expert Software. Results of the treatments were presented for rheometric analysis and viscosity was assessed for the treatments. The optimum treatment (based on the maximum viscosity) for *L. snyderiae* included 65 °C, 35 min and KOH concentration of 7% w v^{-1}. Assessment of viscosity equation and ANOVA analysis were carried out using Design Expert Software, based on the cubicity of the equation. The major aim of this study was to verify occurence of carrageenans in small quantities through primary investigations. However, this study was preliminary and therefore needs further investigations.

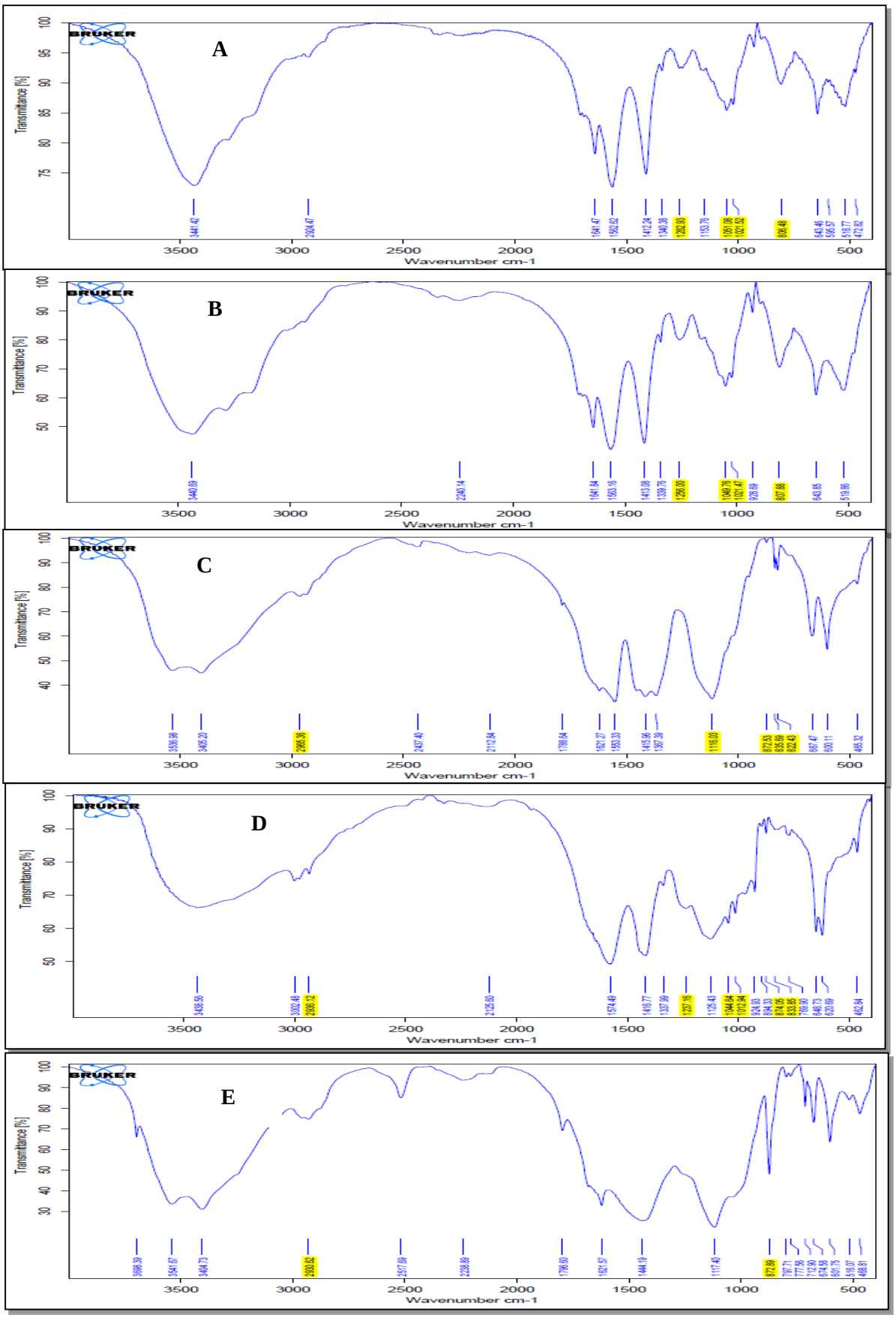

Figure 5. Fourier-transform infrared spectroscopy analyses. A, Fourier-transform infrared spectroscopy analysis of dialysis method; B, Fourier-transform infrared spectroscopy repetition of dialysis method; C, Fourier-transform infrared spectroscopy of calcium hydroxide method; D, Fourier-transform infrared spectroscopy of hydrogen peroxide method; and E, Fourier-transform infrared spectroscopy of autoclave method

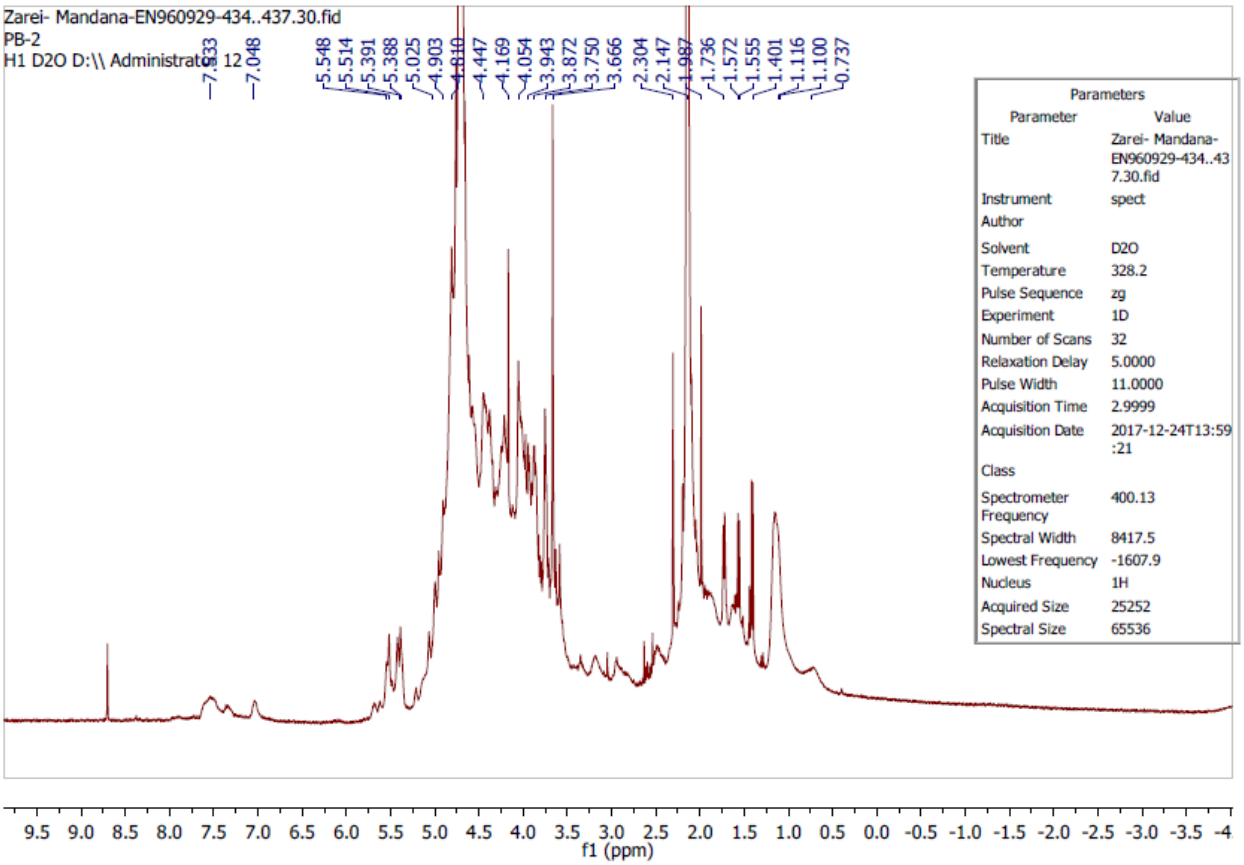

Figure 6. Proton nuclear magnetic resonance of the hydrogen peroxide method

5. Acknowledgements

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6. Conflict of Interest

The authors report no conflicts of interest.

7. Authors Contributions

Conceptualization, Tayebeh Entezari and Ahmad Jamekhorshid; methodology, Mandana zarei and Tayebeh Entezari; software, Mehdi Entezam and Tayebeh Entezari; validation, Mohammad Reza Mohammadizadeh and Mandana Zarei.; investigation, Mandana zarei and Tayebeh Entezari; data curation and original draft preparation, Tayebeh Entezari; writing, review and editing, supervision, project administration and funding acquisition, Mandana Zarei.

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شناسایی و تعیین مشخصات کارگینان استخراج شده از جلبک های قرمز *Laurencia snyderiae* خلیج فارس

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چکیده

سابقه و هدف: کاراگینان در گروهی از جلبک‌های قرمز به‌نام کاراگنوفیت (*Gigartinaceae*، *Solieriaceae*، *Hypneaceae* و *Furcellariaceae*) یافت می‌شود، اما تاکنون وجود این ماده در گونه‌های *Laurencia* مورد بررسی قرار نگرفته است. در این مطالعه، دو گونه *Laurencia* بومی خلیج فارس ، برای استخراج کاراگینان مورد بررسی قرار گرفت. بنابراین، هدف اصلی این مطالعه استخراج، بهینه سازی و خالص سازی کاراگینان از جلبک‌های قرمز *Laurencia snyderiae* بومی خلیج فارس بود.

مواد و روش ها: *Laurencia snyderiae* و *Laurencia papillosa* براساس مشخصات ریخت‌شناسی[۱] آنها شناسایی شدند. طراحی آزمایش با استفاده از نرم‌افزار Design Expert برای تولید و بهینه سازی استخراج کاراگینان‌های نیمه تصفیه شده انجام شد. این نرم‌افزار ۱۸ تیمار را براساس دما، زمان پخت و غلظت KOH برنامه‌ریزی کرد. محصول تیمارها برای آنالیزهای رئومتریک (اندازه‌گیری گران‌روی[۲]) آماده شدند. بهینه‌سازی توسط نرم افزار براساس بیشینه گران‌روی انجام شد. بازده تصفیه کاراگینان با چهار روش استخراج اندازه‌گیری شد. همچنین، طیف‌های به‌دست آمده از طیف بینی مادون قرمز تبدیل فوریه[۳] و طیف سنجی رزونانس مغناطیسی هسته ای[۴] با هم مقایسه شدند. *Laurencia snyderiae* برای بقیه مراحل انتخاب شد.

یافته‌ها و نتیجه‌گیری: براساس تجزیه و تحلیل‌های رئومتریک، محلول نیمه تصفیه‌شده کاراگینان به عنوان یک مایع شبه پلاستیک[۵] غیر نیوتنی شناسایی شد. تیمار بهینه برای *Laurencia snyderiae*، دمای ۶۵ درجه سلسیوس ،۳۵ دقیقه و غلظت ۷ درصد وزنی حجمی برای KOH تعیین شد. نتایج این دو تجزیه و تحلیل نشان داد که کاراگینان‌های تصفیه‌شده به‌دست آمده از *Laurencia snyderiae*، از نوع لامبدا بوده است. بیشترین بازده با روش دیالیز (۳۷٪) به‌دست آمد. با توجه به فراوانی *Laurencia snyderiae* در سواحل خلیج فارس در تمام فصول، مطالعات بیشتر برای به‌دست آوردن کاراگینان با خلوص بیشتر، استفاده از آن‌را در صنایع گوناگون امکان‌پذیر می‌سازد. مطالعات رئولوژیکی گسترده‌تر می‌تواند خصوصیات کاراگینان حاصل را به‌طور دقیق مشخص کند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[۱] morphological
[۲] viscosity
[۳] Fourier transform infrared spectroscopy or FTIR
[۴] nuclear magnetic resonance spectroscopy or NMR
[۵] pseudo-plastic