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Influence of Salt Concentration on Alkaline Extracted Refined Kappa-carrageenan and Its Characterization

Amruth P. [a,b*], Preethy Treesa Paul [a,b], Rosemol Jacob M. [a,b], Jean Mary Joy [a,b], R. Anandan [a] and Suseela Mathew [a*]

[a] Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin-682029, Kerala, India.
[b] Faculty of Marine Sciences, Cochin University of Science and Technology, Cochin-682022, Kerala, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Kappaphycus alverazii remains as a predominant source of kappa-carrageenan which is farmed extensively in Indonesia, Philippines and South East Asia. The current research focuses on the influence of concentration of salts and approach to dialysis on properties of carrageenan. Carrageenan due to its pronounced gelling and viscosifying properties, has gained significant usage in the areas of food, cosmetics, textiles, pharmaceutics, biomedicine, and numerous others. The process of extraction was conducted in a hot, alkaline condition at 80 ^{o}C for a duration of 2 hours. The alkaline substance used was KCl with 2% and 4% concentration and solvent to seaweed ratio was 20:1.Further, the resultant extracted samples were subjected to dialysis to evaluate the effect of dialysis on the quality of carrageenan. The absorbance peak at 849 cm^{-1} from Fourier Transform Infrared-Spectroscopy (FTIR) at all extraction conditions indicated D-galactose-4

**Corresponding author: E-mail: amrithprakash.edu@gmail.com;*

sulphate related to kappa carrageenan obtained with no traces of μ-precursor. The study demonstrated that the concentration of salts and the approach of dialysis have influenced the quality of yield, viscosity, gel strength, moisture content and ash content for the extracted carrageenan, which remains ideal for commercial applications.

Keywords: salt concentration; kappa carrageenan; seaweed; yield and quality.

1. INTRODUCTION

Seaweed gained considerable commercial significance amongst other marine macro-organisms as a result of its effective utilisation as a prosperous raw material for deriving potential bioactives such as lipids, proteins, polyphenols, pigments and polysaccharides [1]. Carrageenan (Fig. 1) from *Kappaphycus alverazii* remains a high-molecular weight, linear, anionic, sulfated galactan extracted from marine red seaweed [2,3]. It constitutes D-galactose and 3, 6-anhydro-galactose units with α-1, 3 and β-1, 4 glycosidic linkages structurally [4]. Carrageenan persists as the most commercially significant seaweed sulphated polysaccharide with an international market value, of US$ 527 million to US$ 626 million from 2009- 2015 [5].

The classification of carrageenan includes λ, κ, ι, ε and μ with regard to the position and number of sulphate moieties of 3, 6 anhydro-galactose content, which influence fundamentally its structural characteristics [3]. It contains an average molecular mass above 100 kDa with sulphate ester about 15 - 40 percentile of its composition. Carrageenan as a seaweed component has gained worldwide market demand as a result of its remarkable gelling, stabilising, emulsifying and viscosifying properties [6]. The predominant functionality of κ-carrageenan is its gel forming potential, even at trace concentrations [7]. In view of this remarkable property, its usage has been expanded from food to various other industries, including textiles, cosmetics, biomedicine and pharmaceutics [8]. Despite this, carrageenan is identified for its pronounced anti-hyperlipidemic (Panlasigui et al. 2003) anti-viral [9], anti-coagulant [10] anti-tumour, and immunomodulatory [11] functionalities.

The higher gel strength and deformality modulus of carrageenan are obtained from hot alkaline treatment at higher temperatures. Moreover, alkaline extraction induces the transformation of native κ-carrageenan from a loosely bonded to a firmly bonded addition compound, which is demonstrated to have enhanced gel forming efficacy [12]. The kappa form of carrageenan is predominantly extracted from *Kappaphycus alverazii*. In this study, a hot alkaline solution (Ca $(OH)_2$) was employed to recover κ-carrageenan from the solution with differential treatments subjected to variations in extraction parameters. Extraction parameters readily influence the quality of extracted carrageenan [13] and yield [14]. The studies of Manuhara et al. [7] have demonstrated that extraction parameters such as concentration of alkali, temperature and duration of heat treatment have been found to influence the amount of 3,6 AG residues produced, and as a consequence of this, the gelling strength of extracted carrageenan is found to increase. Studies also suggest that alkaline solutions are found to remove traces of the sulphate moiety and accelerate the 3, 6-AG formation, which contributes to the augmented gelling potential of the extracted carrageenan [15].

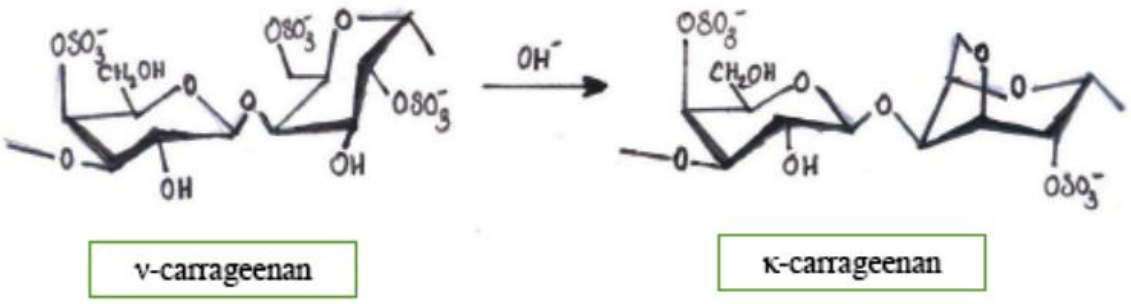

Fig. 1. Structural characteristics of *κ*- carrageenan

In the several reported studies, the extract was not subjected to dialysis, and therefore a higher yield was recorded, but the results remain misleading. Moreover, the extraction of carrageenan with a higher concentration of salts is demonstrated to possess a lower viscosity in comparison to the extraction with lower concentrations. Viscosity highly influences the film forming efficacy of carrageenan and therefore remains critical with regard to its industrial application prospects. Since carrageenan film offers great developmental prospects in the areas of food packaging, wound healing, tissue engineering applications, and numerous others, optimisation of extraction methods remains important to derive carrageenan with the highest yield and superior quality. In the present study, the treatment combination followed a 2% and 4% solution of KCl, algae-to-water ratio of 1:20, and a temperature of 80°C. The samples were subjected to dialysis for purification, and the consequence of the concentration of salts and dialysis on the quality of extracted carrageenan was elucidated.

2. MATERIALS AND METHODS

2.1 Materials

Shade-dried seaweed, *Kappaphycus alverazii* harvested at Madapam Coast, Tamil Nadu, India was used for the study. Supplementary raw materials for the extraction of κ- carrageenan such as distilled water, Ca $(OH)_2$, isopropyl alcohol, 1% HCl, KCl and other materials for analysis, were procured from the local distributor.

2.2. Methods

2.2.1 Carrageenan extraction

100g of the red seaweed was used for the extraction. The samples were subjected to soaking in 5 L of distilled water for 24 h, followed by washing in tap water and distilled water. Then, algal pulp was prepared and then mixed with distilled water at a 1:20 (w/v) ratio, with a slight modification of the method of Manuhara et al. [7]. The mixture was made alkaline (pH ± 9) by adding freshly prepared $Ca(OH)_2$ solution, and the extraction was carried out at 80°C for 2 h by stirring at 750 rpm. Further, on neutralisation with a 1% HCl solution, the pH changed to 7 and the solution was heated at 90 °C for 30 minutes with continuous stirring. The mixture was sieved, the supernatants collected, washed with isopropyl alcohol, and centrifuged. The separation of liquid parts is ascertained by filtration. The filtrate was allowed to coagulate by using 2% and 4% KCl at consecutive steps while maintaining the filtrate and KCl solution ratio of 1:1 with continuous stirring for 15 minutes at 750 rpm, and then the resultant was filtered to isolate gel from water. The gel was collected and subjected to dialysis through a 14 KDa membrane for 48 hours. Further, the sample was dried by lyophilization for 48 h and stored at room temperature for further analysis.

2.2.2 Analysis

Dialysis was performed to achieve purity in samples through the removal of excess salts. For the removal of salts, the samples were passed through a 14 KDa membrane. The yield of the extracted carrageenan was calculated by dividing the weight of the carrageenan by the weight of the dried algae. Brookfield viscometer was employed to measure the viscosity of treated samples as κ- Carrageenan remains a thermoreversible non-Newtonian fluid. For evaluating the viscosity, 1.5% of the carrageenan solution was subjected to heating up to 80 °C on a hot plate with constant stirring, and further viscosity was measured with the aid of Spindel. For the evaluation of gelling strength, a carrageenan solution was prepared, and then the solution was analysed with the aid of the Testing Machine MPY (PA-104-30). The percent of moisture and ash content should comply with the FDA standard for food technological and pharmaceutical applications. Moisture and ash content were determined by the gravimetric method [16]. FTIR was employed to confirm the polyelectrolyte interaction in the samples. For the characterization of the sample, infrared spectroscopy was performed in an FTIR spectrophotometer, where the spectra bands were recorded in the bandwidth of 4000 to 400 cm–1.

2.2.3 Statistical analysis

In each treatment, two replications were employed for the statistical analysis, and analytical evaluations were performed using SPSS for Windows (Version 19). The differences in mean ± values amongst different samples were determined by employing one-way analysis of variance (ANOVA) and the Duncan Multiple Range Test.

3. RESULTS AND DISCUSSION

3.1 Alkaline Extraction

Alkaline extraction aids the cyclization reaction with hydroxide (-OH) to generate the 3, 6-anhydro-bridge. The presence of 6-sulfated-a galactose units is a prerequisite for the reaction to occur. In the process, the sulfated polysaccharide is heated in strong alkaline media, and ionisation of the free 3-OH group is necessary to generate an intramolecular nucleophilic displacement of the sulphate group at position 6. Bivalent ions play a predominant role in the generation of helix formation. In the present study, potassium ions, which have the potential to be introduced between double helices and promote charge neutralisation of sulphate groups and stabilisation of the double helix, were used. A similar effect is not induced by ions, which are considerably bigger (hydrated radius). Additionally, divalent cations possess the potential to decrease the viscosity of extraction at higher concentrations and increase it at lower concentrations (Campo et al., 2009). In the current study, κ-carrageenan derived by alkaline extraction with an extraction condition of 4% KCl has contributed to the increased yield and the decrease of moisture, ash viscosity, and gel strength for samples. The carrageenan viscosity demonstrated decreased value due to a higher KCl concentration (4%) and increased value at a lower concentration (2%).

When the samples were subjected to dialysis, approximately 10% of the reduction in yield and fluctuations in parameters including viscosity, gelling strength, moisture and ash content occurred. Dialysis has induced considerable fluctuation in yield, viscosity, gelling strength, moisture and ash values despite the variable concentration of salts (Table 1).

3.2 Yield

Table 1 depicts the effect of the properties of extracted carrageenan on different concentrations of KCl before and after dialysis. The yield of the extracted carrageenan was determined by weighing the carrageenan and subjecting it to 80 mesh sieving. The calculated yield was 19.53% to 29.45 % for samples without dialysis. In the study of Manuhara et al. [7], *κ*-carrageenan derived by hot alkaline treatment with 1.5% concentration of KCl has approached a similar yield. The difference in yield is due to the fact that the dialysis has removed excess salts from the sample. In the study of Al-Alawi et al. [3], the yield obtained was found to be very low (12.69%) for the extraction conditions (6% NaOH, 3.5 h, and 80°C) in comparison to the yield (33.2%) previously reported for the similar plant collected from a similar geographical area and following an extraction process nearly under similar conditions (6% NaOH, 4 h, and 80–85°C). This significant difference in yield was noticed since there was no dialysis step in the previous study. Therefore, the current study focused on optimising the extraction process for maximum yield and superior quality, and it has been demonstrated that carrageenan yield is significantly increased with an increase in alkali treatment strength.

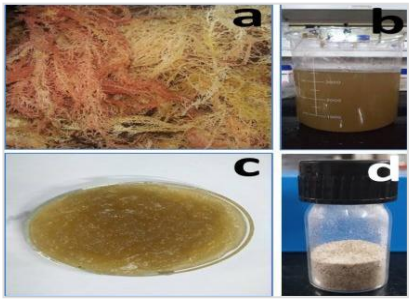

Fig. 2. (a) Red Seaweed *Kappaphycusalverazii*, (b) & (c) Carrageenan gel obtained from the seaweed by hot alkali treatment (d) dried and powdered carrageenan

3.3 Viscosity

The highest viscosity is observed for samples extracted at a 2% KCl concentration for both the non-dialyzed and dialyzed samples. The salts are found to decrease the viscosity of carrageenan by reducing the electrostatic repulsion between sulphate groups [17]. In the current study, 7.30cP was observed at extraction of the sample at 2% KCl concentration, and 6.56cP was observed at extraction of the sample at 4% KCl concentration. A difference in viscosity is observed on treatment at different salt concentrations; on increasing the salt concentration, viscosity is observed to reduce. This reduction in viscosity might be due to the electrostatic repulsion between the sulphate groups induced by the salts. A similar result was observed in the study of Arfini [18], where it was demonstrated that carrageenan viscosity was higher in treatment with 1% KCl in comparison to treatment with 1.5% KCl. In the current study, the carrageenan viscosity met the minimum standard

Table 1. Properties of extracted carrageenan

Properties	Before dialysis		After dialysis	
Concentration KCl	2%	4%	2%	4%
Yield (%)	19.53 ± 1.23^{b}	29.45 ± 1.65^{c}	15.62 ± 1.06^{a}	25.45 ± 1.53^{c}
Viscosity(cP)	7.30 ± 0.20^{b}	6.56 ± 0.21^{a}	7.50 ± 0.10^{b}	6.76 ± 0.15^{a}
Gel strength (g/cm^2)	99.20 ± 2.87^{c}	92.80 ± 1.13^{b}	96.70 ± 1.23^{c}	88.63 ± 0.93^{a}
Moisture (%)	6.76 ± 0.17^{c}	5.30 ± 0.10^{b}	5.26 ± 0.06^{b}	4.19 ± 0.09^{a}
Ash (%)	60.38 ± 2.43^{b}	$57.51 \pm 2.08^{a,b}$	$59.71 \pm 2.38^{a,b}$	55.74 ± 1.95^{a}

The values are expressed as mean ± SD and multiple comparisons were analysed by one-way analysis of variance (ANOVA) followed by the Duncan post hoc test.
Different letters (a-c) in a column denote significant differences ($P < 0.05$)

(5cP) of the FAO. The viscosity of food-grade carrageenan is recommended in the range of 5cP to 800cP [4]. In our study, fluctuating values of viscosity were observed in samples before and after dialysis. This clearly indicates that dialysis has reduced the number of salts in the sample and interfered with electrostatic repulsion amongst sulphate groups to generate fluctuating viscosity values. Since viscosity is an important rheological parameter for the application of carrageenan, optimisation of extraction parameters gains critical significance. Since the amount of salt present in the extracted sample has a close association with viscosity, dialysis plays a predominant role in altering the viscosity of the resultant samples by eliminating the excess salt content in them.

3.4 Gel Strength

Gelling strength is an important parameter with regard to carrageenan application in various fields. The gelling properties of carrageenan have significance in the areas of food, food technology, packaging, cosmetics, pharmaceutics and numerous others [19]. Recently, κ-carrageenan has gained great developmental prospects in the area of wound dressing application due to its remarkable gelling potential. In light of these, optimisation of extraction parameters to augment the gelling strength by following the regulations of FAO remains an important concern. The κ-carrageenan gel strength increases with the increase in concentration of K+ and Ca^2+ cations on addition of chloride salt [20,4]. The extraction of κ-carrageenan in the presence of K+ remains ideal to increase the gelling strength. The atomic size of K+ is higher in comparison to Ca^2+ and Na+; therefore, K+ possesses higher penetration power within the molecular structures to ascertain increased gelling strength. The gel strength is directly proportional to the concentration of carrageenan and salts. The higher concentration of potassium salts is found to produce a weaker gel strength. In the current approach, the trend of decreasing gelling strength is observed when the concentration of KCl is increased. Albeit, extraction was conducted at 2% and 4% concentrations of KCl, a significant difference in values was not observed. This mild fluctuation may not affect the gelling efficacy of carrageenan for application prospects.

3.5 Moisture

The moisture content of the extracted carrageenan ranged from 5.26% to 4.19%. Fluctuations in the value of moisture were observed on approaching dialysis and in the differential concentration of KCl. Moreover, the moisture content of all carrageenan samples met the FAO standard. In comparison to the results of studies conducted by Manuhara et al. [7] and Herliany [21], the moisture content of the current study demonstrated a lower value.

3.6 Ash

A high concentration of KCl solution might cause a high ash content in carrageenan. High KCl concentrations cause high levels of kalium. The ash content of dried *K. alvarezii* harvested in Mandapam, Tamil Nadu is 27.54%. The ash content of the carrageenan (59.71% to 55.74%) satisfies the FAO recommended standard (15% to 40%).

3.7 FTIR

Fig. 3 shows the FTIR spectrum of carrageenan extracted by 2% and 4% KCl solutions. The absorption peaks correspond to sulphate ester (1234 cm^{-1}), glycosidic linkage (1072 cm^{-1}), 3, 6-anhydro-d-galactose (926 cm^{-1}), and D-galactose-4-sulphate (849 cm^{-1}). The former peak, which was also observed at 840 cm^{-1} to 850 cm^{-1} in other studies [22,23], indicated that the carrageenan was κ-type.

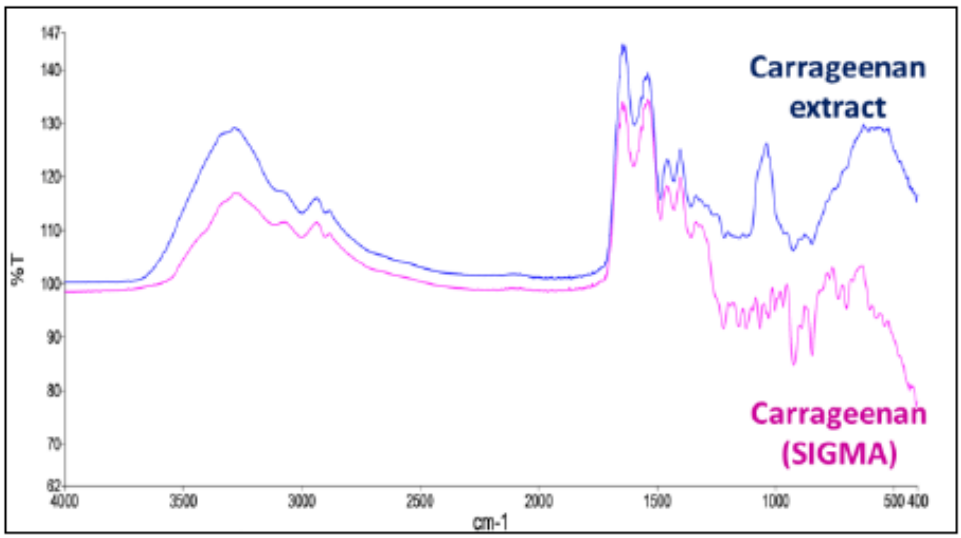

Fig. 3. FTIR spectra of extracted carrageenan compared with carrageenan from SIGMA depicting similar spectra

4. CONCLUSION

In the current study, parameters such as the effect of concentration of salts and the approach of dialysis on properties like yield, viscosity, gelling strength, moisture and ash of κ-carrageenan are evaluated, along with the ideal conditions that can be applied for the extraction of carrageenan from *Kappaphycus alverazii* to eliminate excessive processing that may lead to deterioration of the molecule and compromise its yield and quality. The FTIR spectra demonstrated the presence of *κ*- carrageenan, to no extent, or minor quantities of μ or *ι*-carrageenans in the evaluated samples, which verify the effectiveness of the alkaline extraction and mild parameters for total conversion achievement. The current approach aids in obtaining carrageenan with superior yield and quality optimal for commercial applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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