Effect of salt concentration and dialysis on properties of alkaline extracted refined kappa-carrageenan and its characterization

ABSTRACT

Kappaphycus alverazii remain as a predominant source of kappa-carrageenan which is farmed extensively in Indonesia, Philipines and South East Asia. The current research focus on the influence of concentration of salts and approach of dialysis on properties of carrageenan such as yield, gel strength, viscosity, moisture and ash. Carrageenan due to its pronounced gelling and viscosifying properties have gained significant usage in the area of food, cosmetics, textiles, pharmaceutics biomedicine, and numerous others. Carrageenan with good gelling strength is ideal for commercial application, which are chiefly obtained from hot alkaline extraction process. The process of extraction was conducted at hot alkaline condition at 80 ^{o}C for a duration of 2 hrs. Alkaline substance used was KCl with 2% and 4 % concentration and solvent to seaweed ratio was 20:1. Further, the resultant extracted samples was subjected to dialysis to evaluate the effect of dialysis on quality of carrageenan. The study demonstrated best value of yield, viscosity, gel strength, moisture content and ash content for the extracted carrageenan, which remains ideal for commercial applications. The study concluded that the concentration of salts and the approach of dialysis have influenced the quality of the yield, viscosity and gel strength of the extracted carrageenan. The absorbance peak at 849 cm^{-1} from Fourier Transform Infrared-Spectroscopy (FTIR) at all extraction conditions indicated D-galactose-4 sulphate related to kappa carrageenan obtained with no traces of μ-precursor.

1. Introduction

Seaweed gained considerable commercial significance amongst other marine macro-organisms as a result of its effective utilization as a prosperous raw material for deriving potential bioactives such as lipids, proteins, polyphenols, pigments and polysaccharides (Muhamed *et al.,* 2012). Carrageenan remains as a high molecular weight, linear, anionic, sulfated, galactan extracted from marine red seaweed (Estevez *et al.,* 2000; Al-Alawi *et al.*, 2011). It constitutes D-galactose and 3, 6-anhydro-galactose units with α-1, 3 and β-1, 4 glycosidic linkages structurally (Necas & Bartosikova, 2013). 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 (Rhein-Knudsen et al., 2015).

Classification of carrageenan include λ, κ, ι, ε, μ on regard to the position and number of sulfate moieties of 3, 6 anhydro-galactose content, which influence fundamentally its structural characteristic (Al-Alawi*et al*., 2011). It contains average molecular mass above 100 kDa with sulphate ester about 15 - 40 percentile in composition. Carrageenan as a seaweed component have gained world-wide market demand as a result of its remarkable gelling, stabilizing, emulsifying and viscosifying properties (Quin *et al*., 2018). The predominant functionality of κ-carrageenan is its gel forming potential even at trace concentrations (Manuhara *et al.,* 2016). In view of this remarkable property, its usage has been expanded from food to various other industries including textiles, cosmetics, biomedicine and pharmaceutics (Suganya *et al*., 2016).

Despite, carrageenan is identified for its pronounced anti-viral (Campo *et al*., 2009) anti-tumor, immunomodulatory (Pacheco et al., 2020, anti-hyperlipidemic (Panlasigui *et al*., 2003) and anti-coagulant functionalities (Yermak et al., 2012).

The higher gel strength and deformality modulus of carrageenan is obtained from hot alkaline treatment at higher temperature. Moreover, alkaline extraction induces transformation of native κ-carrageenan from loosely bonded to firmly bonded addition compound which are demonstrated to have enhanced gel forming efficacy (Rortbart *et al*., 1988). The kappa form of carrageenan is predominantly extracted from *Kappaphycus alverazii*. In this study, 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 (Heriyanto et al., 2018) and yield (Al-Nahidi et al., 2019). The studies of Manuhara et al., 2016 have demonstrated that extraction parameters such as concentration of alkali, temperature, duration of heat treatment have found to influence the amount of 3,6 AG residues produced and as a consequence of this, gelling strength of extracted carrageenan is found to increase. Studies also suggest that alkaline solution are found to remove traces of the sulphate moiety and accelerates the 3,6-AG formation that contributes to augmented gelling potential of the extracted carrageenan (Yasita, 2009).

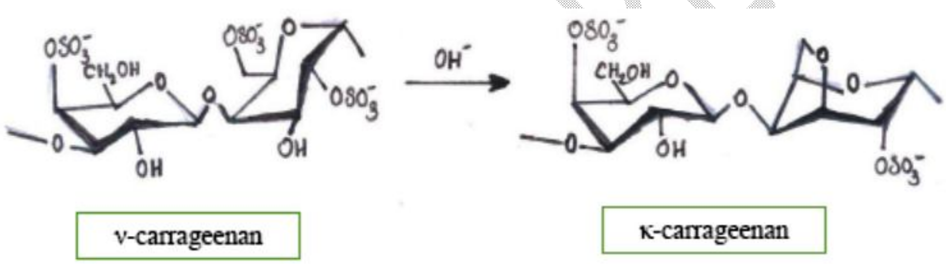

Fig.1. Structural characteristics of *κ*- carrageenan

In the several reported studies, the extract was not subjected to dialysis and therefore higher yield were recorded and the results remains misleading. In the present study, approximately 10 % of difference in yield was observed on subjecting the samples to dialysis. Moreover, the extraction of carrageenan with higher concentration is demonstrated to possess lower viscosity on comparison to extraction with lower concentration of salts. Viscosity highly influences the film forming efficacy of carrageenan and therefore remain critical with regard to its industrial application prospects. Since carrageenan film offers great developmental prospects in the area of food packaging, wound healing and tissue engineering applications, and numerous others, optimization of extraction methods remains important to derive carrageenan with highest yield and superior quality.

In the present study, we have approached the treatments combination following 2% and 4% solution of KCl, algae to water ratio (1:20), and temperature of precipitation at 80 °C. The researches on influence of dialysis on the rheological attributes of kappa carrageenan have never been determined. Therefore, in the current approach, the samples were subjected to dialysis for purification and the consequence of concentration of salts and dialysis on the quality of extracted carrageenan was elucidated.

2. Material and Methods

2.1. Materials

Shade dried seaweed; *Kappaphycus alverazii* harvested at Madapam Coast of 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 also 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 distilled water for 24 h followed by through washing in tap water and distilled water. Then, algal pulp was prepared and then mixed with distilled water at 1:20 (w/v) ratio with slight modification of the method of Manuhara *et al.*, 2016. The mixture was converted to 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 the mixture was converted to pH 7 on neutralization with 1 % HCl solution and heated at 90 °C for 30 min for continuous stirring. Then, the mixture was sieved, the supernatants was collected, washed with isopropyl alcohol and centrifuged. The separation of liquid part is ascertained by filtration. The filtrate was allowed to coagulate by using 2 % and 4 % KCl at consecutive steps by maintaining filtrate and KCl solution ratio 1: 1 with continuous stirring for 15 min at 750 rpm, and then the resultant was filtered to isolate gel from water. The gel was collected and subjected to dialysis by passing them through a 14 KDa membrane. The resultant gel was subjected to dialysis for 48 hrs. Further, the sample was dried employing lyophilization for 48 h and stored at room temperature for further analysis.

2.2.2. Analysis

The calculation of yield was done on dividing the carrageenan weight by dried algae weight. *κ*- Carrageenan remain as a thermoreversible non-Newtonian fluid and evaluation of viscosity and gelling strength remain critical for its application in multitude of sectors. Viscometer Brookfield was employed to measure the viscosity of treated samples. For evaluating the viscosity, 1.5 % of carrageenan solution was subjected to heating on employing hot plate oven and stirring was followed until 80 °C, further viscosity was measured with the aid of Spindel. For the evaluation of gelling strength, carrageenan solution was prepared and then the solution was analyzed with the aid of Testing Machine MPY (PA-104-30). The percent of moisture and ash content should comply the FDA standard for food technological and pharmaceutical applications. Moisture and ash content were determined by gravimetric method (AOAC, 1990). Dialysis was performed to achieve purity in samples by the removal of excess salts in the sample. For the removal of salts, the samples were passed through a 14 KDa membrane. FTIR was employed to confirm the polyelectrolyte interaction in the samples. For the characterization of the sample, infrared spectroscopy was performed in FTIR spectrophotometer wherein the spectra bands were recorded in the bandwidth of 4000 to 400 cm^{-1}.

2.2.3. Statistical analysis

Duplicates were performed for all treatments and analytical evaluations. SPSS for windows (version 19) was employed for the statistical analysis. The differences of mean ± values amongst different sample were carried out on employing one-way analysis of variance (ANOVA) and 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-sulphated-a galactose units is a requisite for the reaction to occur. In the process, the sulphated polysaccharide is heated in strong alkaline media and ionization of free 3-OH group is necessary to generate an intramolecular nucleophilic displacement of the sulphate group at position 6. Bivalent ions has predominant role for the generation of helix formation. In the present study, we approached potassium ions, having the potential to be introduced between double helices and they promote charge neutralization of sulphate groups and stabilization of double helix. Similar effect is not induced by ions which are considerably bigger (hydrated radius). Additionally, divalent cations possess the potential to decrease the viscosity on extraction with higher concentration and increases at lower concentrations (Campo et al., 2009). In the current study, κ-carrageenan derived by alkaline extraction with extraction condition 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 higher KCl concentration (4%) and increased value at lower concentration (2%).

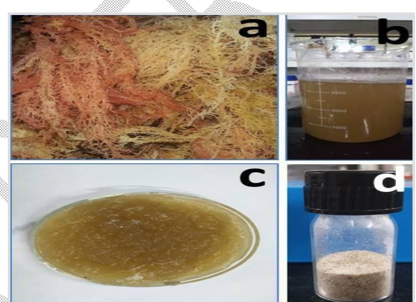

Fig 2. **(**a) Red Seaweed *Kappaphycusalverazii*, (b) & (c) Carrageenan gel obtained from the seaweed by hot alkali treatment (d) dried and powdered carrageenan.

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

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 ± 2.08[a,b]	59.71 ± 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 ($P < 0.05$).

3.2. Yield

Table 1 depicts the effect of properties of extracted carrageenan on different concentration of KCl and before and after dialysis. The yield of the extracted carrageenan was determined on weighing carrageenan on subjecting to 80 mesh sifting. The calculated yield was 19.53 % to 29.45 % for samples without dialysis. In the study of Manuhara et al., (2016), κ-carrageenan derived by hot alkaline treatment with 1.5 % concentration of KCl has approached similar yield. The difference in yield is found since the dialysis has removed excess salts from the sample. In the study of Al-Alawi et al.,2011 the yield obtained is found very low (12.69%) for the extraction condition (6% NaOH, 3.5h, and 80∘C) on comparison to the yield (33.2%) previously reported for the similar plant collected from the similar geographical area and followed extraction process nearly under similar conditions (6% NaOH, 4h, and 80 85°C). This significant difference in yield is noticed since there performed no dialysis step in the previous study. Therefore, the current study has focused to approach an optimization at extraction process for achieving maximum yield with superior quality. Therefore, in the current approach it has been demonstrated that carrageenan yield significantly reduced with the increase in alkali treatment strength.

3.3 Viscosity

The highest viscosity is observed for sample extracted at 4 % KCl concentration for both the samples non-dialyzed and dialyzed. The salts are found to decrease the viscosity of carrageenan by reducing the electrostatic repulsion between sulphate groups (Moirano, 1977). In the current study, 7.30 cp was observed at extraction of sample at 2 % KCl concentration and 6.56 cP was observed at extraction of sample at 4 % KCl concentration. Difference in viscosity is observed on treatment at different salt concentration, on increasing the salt concentration, viscosity is observed to reduce. This reduction of viscosity might be due to the electrostatic repulsion between the sulphate groups induced by the salts. Similar result was observed in the study of (Arfini, 2011), it has been demonstrated that carrageenan viscosity observed higher on treatment with 1 % KCl on comparison to treatment with 1.5 % KCl. In the current study, the carrageenan viscosity has met minimum standard (5 cP) of FAO. The viscosity of food-grade carrageenan is recommended in the range 5 cP to 800 cP (Necas and Bartosikova, 2013). In our study, fluctuated values of viscosity are observed in sample before and after dialysis. This clearly indicates that dialysis has reduced the number of salts in the sample and interfered in electrostatic repulsion amongst sulphate groups to generate fluctuated viscosity values. Since viscosity is an important rheological parameter for the application of carrageenan, optimization of extraction parameters gains critical significance. Since the amount of salts present in the extracted sample has a close association with viscosity, dialysis plays a predominant role in altering the viscosity of 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. Gelling properties of carrageenan has significance in the area of food, food technological, packaging, cosmetics, pharmaceutics and numerous others (Sedayu *et al*., 2019). Recently, k-carrageenan has gained great developmental prospects in the area of wound dressing application due to its remarkable gelling potential. In light of these, optimization of extraction parameters to augment the gelling strength by following the regulations of FAO remains an important concern. The κ-carrageenan gel strength increases as the increase of concentration of K^+ and Ca^{2+} cation on addition of chloride salt (Doyle *et al*.,2002; Nekas and Bartosikova, 2013). The extraction of κ-carrageenan in the presence of K^+ remain ideal to increase the gelling strength. The atomic size of K^+ is higher on comparison to Ca^{2+} and Na^+, therefore, K^+ possess 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 decrease in gelling strength is observed when concentration of KCl is increased. Albeit, extraction conducted at 2 % and 4 % concentration of KCl, significant difference in values is 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 was 5.26 % to 4.19 %. Fluctuations in value of moisture was observed on approaching dialysis and differential concentration of KCl. Moreover, the moisture content of all carrageenan samples met the FAO standard. On comparison to the results of study conducted by (Manuhara *et al*., 2016; Herliany, 2011), the moisture content of current study demonstrated lower value.

3.6. Ash

High concentration of KCl solution might cause high ash content of the carrageenan. High KCl concentration cause high content of kalium. The ash content of dried *K. alvarezii* harvested in Mandapam coast of 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 showed FTIR spectrum of carrageenan extracted by 2 % and 4 % KCl solution. 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 (Dewi *et al*., 2015; Webber *et al*., 2012), indicated that the carrageenan was κ-type.

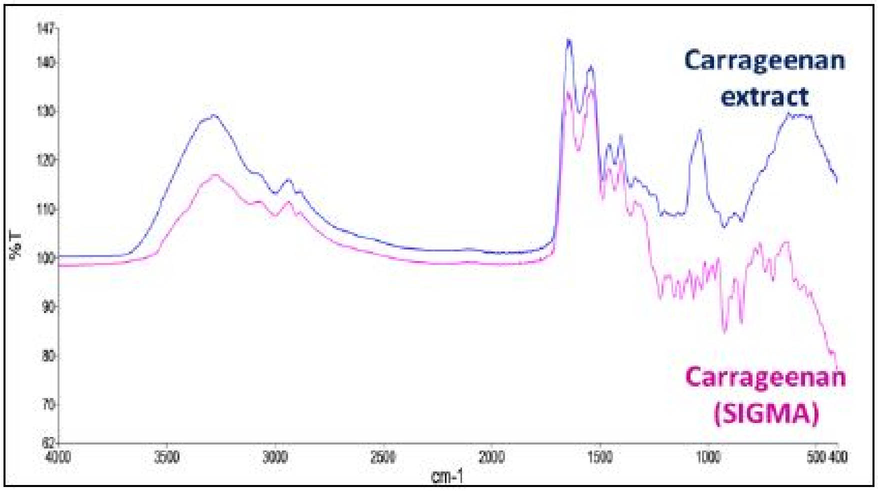

Fig 3. FTIR spectra of extracted carrageenan compared with carrageenan from SIGMA depicting similar spectra.

Conclusion

We have studied the parameters such as effect of concentration of salts and the approach of dialysis on properties like yield, viscosity, gelling strength, moisture and ash of k-carrageenan and evaluates 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 molecule and compromise its yield and quality. The FTIR spectra demonstrated the presence of κ- carrageenan, with 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 to derive carrageenan with superior yield and quality optimal for commercial applications.

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