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Effect of alkaline treatment on the sulfate content and quality of semi-refined carrageenan prepared from seaweed *Kappaphycus alvarezii* Doty (Doty) farmed in Indian waters

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Seaweed *Kappaphycus alvarezii* previously known as *Eucheuma cottonii* is one of the best sources of kappa carrageenan and is cultivated in Philippines, Indonesia, Malaysia and other countries including India. In the present study, semi-refined carrageenan (SRC) was prepared from *K. alvarezii* with different concentrations of KOH (6, 12, 18 and 24%) at 80 ± 2°C for 2 h; its sulfate contents were 13.73±0.74, 13.66±0.27, 13.84±0.66 and 14.76±0.18%, respectively, and it was 14.10±0.34% for untreated clean weed (control). The sulfate removal with increasing concentration of KOH used for processing SRC is not statistically significant ($p < 0.05$). The KCl gel strength of untreated clean weed and 6, 12, 18 and 24% KOH treated weed was 97±10.27, 650±12.11, 637±10.25, 552±18.71 and 526±26.55 g cm^{-2}, respectively. Contents of 3,6-anhydrogalactose were 28.30±0.52, 33.41±0.50, 32.97±0.42, 31.15±0.60 and 31.61±0.17%. FTIR spectroscopy showed that molecules in all four SRC samples are quite similar. Spectral band was at 1257 cm^{-1} which referred to ester sulfate of 930 cm^{-1} for 3,6 anhydrogalactose and 848 cm^{-1} assigned to galactose-4-sulfate. From the present investigation, it was observed that sulfate removal and quality improvement with increasing concentration of KOH for cooking seaweed is not statistically significant; therefore, seaweed can be subjected to lower concentration of KOH treatment (6 to 12%) to produce semi-refined carrageenan on commercial scale.

Key words: Seaweed, *Kappaphycus alvarezii,* KOH treatment, semi-refined carrageenan (SRC), sulfate content, FTIR spectra, gel strength.

INTRODUCTION

Carrageenans are sulfated linear polysaccharides extracted from certain red seaweeds of the class Florideophyceae. They have been extensively used in the food industry as thickening and gelling agent and more

recently used in pharmaceutical industry as excipient in pills and tablets (Campo et al., 2009; Aguilan et al., 2003). Since natural carrageenans are mixtures of different sulfated polysaccharides, their composition differs from batch to batch. Carrageenans from particular seaweed species and geographic districts differ considerably in their structure and rheological properties of solutions and gels. Therefore, the quantitative analysis of carrageenan or semi-refined carrageenan (SRC) batches is of greatest importance for industry to deliver a standard product and to develop new application based on their unique intrinsic properties. Farming of tropical seaweed *Kappaphycus alvarezii* Doty (Doty) (previously known as *Eucheuma cottonii*) in Indian water has been going on for more than a decade (Krishnan and Narayanan, 2010), and *Kappaphycus* is extensively used for the industrial production of κ-carrageenan (Glicksman, 1983; Hoffmann et al., 1993). The industrial pure carrageenan manufacturer is no longer limited to extraction of carrageenan in pure form; nowadays, SRC is used as an alternative to carrageenan. Earlier, SRC was mainly used in pet food and other applications where clarity was not an important criterion. However, recently there has been improved method to produce SRC of food grade for human consumption.

Further, the product was also processed by applying food safety procedures; therefore SRC is permitted in human consumption (Philips, 2002; Mehta et al., 2008; van de Velde et al., 2002). Two chemical transformations occur when the carrageenan containing seaweed is subjected to cooking in the presence of KOH at elevated temperature. The first transformation is desulfation, which occurs when a sulfate group bounded to the 6-position of the galactose (3,6-AG) units of a carrageenan polymer molecule is removed by the K^+ ions to form K_2SO_4 in the cooking solution. The second reaction is a dehydration of the de-sulfated product to create the recurring 3,6 anhydrous galactose polymers (Mehta et al., 2008; Christopher and Michael, 1998). It is reported that during the alkaline treatment and further washing of carrageenan bearing seaweed, alkali soluble and cold water soluble material are removed (Hoffmann et al., 1993; McHugh, 2003).

The aim of the present study was to prepare SRC with different concentrations of KOH, check the degree of desulfation with increasing concentration of KOH and observe any significant improvement in the quality of the SRC product.

MATERIALS AND METHODS

Sampling of *Kappaphycus alvarezii*

K. alvarezii fresh material was sampled from commercial farming site at Mandapam (9.28°;N 79.12°E), India; foreign matter was removed from it and then dried under sun. Dry weed samples (35% moisture) were cut into small pieces (≈1" inch), mixed well by coning and quartering method and used for preparation of SRC.

Preparation of semi-refined carrageenan

The dry-weed sample was soaked in water for 30 min (1 part of seaweed to 6 parts of water) to remove sand and salt and given consecutive second water wash for 10 min. Then, the washed material was cooked at 80 ± 2°C for 2 h at different KOH levels (6, 12, 18 and 24%). Cooked samples were then washed with tap water at 1: 2.5 ratio (w/v) to remove the excess KOH excess and then sun-dried. The washed seaweed without KOH treatment was dried and treated as control sample. After that, the test samples were micronized into powder, and sieved through 80# mesh (180 A.S.T.M or 80 μm) to obtain SRC samples. Yield was calculated as ratio of dried SRC weight to dried seaweed weight. KOH level in spent liquor, post washes and final products of SRC were tested to see check its consumption level during SRC process. Five replications in each experiment were conducted and data were used to interpret the results.

Quality parameter analysis

Ash content was determined gravimetrically by making ash in muffle furnace at 550°C (PNS 601:2011, Carrageenan and Processed *Eucheuma* seaweeds - Specifications). Ester sulfate content was determined using the method of sulfate hydrolysis followed by precipitation of sulfate as $BaSO_4$ (JECFA, 2010). A known amount of dried SRC (W1, g) was hydrolyzed with 50 mL of 1 N HCl for 30 min at its boiling temperature and 10 mL of 0.25 M $BaCl_2$ was dropped into it. After cooling at room temperature for 5 h, the barium sulfate precipitates were filtered using ashless filter paper and incinerated for 1 h at 700°C. The white ash was weighed as W2 and sulfate content was calculated using the equation below:

% sulfate = (W2/W1) x 100 x 0.4116

Acid insoluble matter was determined using 0.1% sulfuric acid (PNS 601:2011, Carrageenan and Processed Eucheuma seaweeds - Specifications). Sodium and potassium contents were determined by flame photometry method. The FT-IR spectra of all samples were analyzed in KBr pellets using FT-IR Spectrophotometer (Perkin-Elmer Spectrophotometer GX). Viscosity was measured at 1.5% in water at 75°C, 30 rpm and spindle no.62 using Brookfield LVDV-II+pro. KCl gel strength was determined by making 1.5% SRC in 0.2% KCl solution using Brookfield Texture Analyze, Model CT3 4500 and water gel strength was measured by preparing 1.5% SRC solution in water. The 3,6-anhydrogalactose was estimated by improved phenol-resorcinol method using fructose as standard (Yaphe and Arsenault, 1965). Microbial profile of SRC samples was carried out using the procedure as described by Jarvis et al. (1977) and Cruchaga et al. (2001).

Statistical analysis

Statistical analyses such as analysis of variance (ANOVA, SYSTAT version 7), correlation and regression were applied to analysis the data of sulfate contents and it was considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the KOH level in spent liquor, post washes and final product of SRC samples. The KOH level in spent liquor of 6, 12, 18 and 24% KOH treated material was 4.65±0.12, 9.92±0.12, 14.86±0.95 and 20.12±0.57%, respectively; similarly, the level of KOH in the first and

Table 1. KOH residues in spent and post wash waters and final product of semi-refined carrageenan

Concentration of KOH used for preparation of SRC (%)	KOH residues (%)			
	Spent liquor	1st wash water	2nd wash water	Final Product (SRC)
6	4.65±0.12	1.72±0.13	0.59±0.00	1.27±0.11
12	9.92±0.12	3.66±0.58	1.11±0.01	1.88±0.05
18	14.86±0.95	4.74±0.33	1.57±0.03	3.75±0.05
24	20.12±0.57	6.85±1.02	2.28±0.018	4.90±0.05

Table 2. Quality and Physicochemical properties of semi-refined carrageenan obtained by processing in different KOH concentration

Parameters	Conc. of KOH used for preparation of SRC (%)				
	Control	6	12	18	24
Yield (%); ±SD					
Sulfate (%); ±SD	14.10±0.34	13.73±0.74	13.66±0.27	13.84±0.66	14.76±0.18
Acid insoluble matter (%); ±SD	13.14±0.12	13.87±0.08	13.77±0.07	14.50±0.08	14.84±0.11
Ash (%); ±SD	27.87±0.04	26.07±0.16	26.87±0.04	28.04±0.03	29.05±0.22
K^+ (%);±SD	6.19±0.36	7.12±0.10	10.54±0.96	14.91±1.02	17.33±1.83
Na^+ (%):±SD	2.15±0.04	0.40±0.38	0.32±0.04	0.267±0.01	0.25±0.01
3,6-anhydrogalactose (%); ±SD	28.30±0.52	33.41±0.50	32.97±0.42	31.15±0.60	31.61±0.71
KCl gel strength (g cm^{-2})	97±10.27	650±12.11	637±10.25	552±18.71	526±26.55
Water gel strength (g cm^{-2})	Nil	310±10.33	286±17.19	235±14.4	224±11.82
Viscosity (cP)	104.80±0.72	49.95±0.21	34.84±0.75	18.67±0.54	13.62±0.42
Total plate count (TPC)	☐ 5000	☐ 2500	☐ 2500	☐ 2500	☐ 2500
Yeast and mould	☐ 300	☐ 100	☐ 100	☐ 100	☐ 100
Salmonella and Shigella	Negative	Negative	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative	Negative	Negative

second post washes and final product increased with increasing concentration of KOH used for cooking (Table 1). Higher KOH level (>2%) in the final SRC products obtained from treating with 18 and 24% shows that further washes are required to remove the excess KOH. Table 2 depicts the quality of SRC prepared with different conc. of KOH. The yield of SRC samples obtained from treating with KOH concentration of 6, 12, 18 and 24% was 31.72±1.35, 32.55±1.35, 30.46±1.56 and 29.89±0.92% and yield of untreated material was 34.42±0.44%. Comparatively, higher yield (2.6 to 8.8%) was obtained from material treated with 12% KOH compared to low (6% KOH) and higher KOH conc. (18 and 24%). This observation is in line with the literature reported earlier (Iain, 2008). The sulfate contents of SRC prepared from 6, 12, 18 and 24% KOH were 13.73±0.74, 13.66±0.27, 13.84±0.66 and 14.76±0.18%, respectively, with 14.10±0.34% in untreated material. There are many reports on increasing level of desulfation with increasing concentration of KOH used for cooking carrageenan bearing seaweed (Sperisa et al., 2011). But in the present investigation, it was observed that sulfate removal with increasing concentration of KOH used for processing SRC is not statistically significant. The reason

for more sulfate in untreated clean weed could be due to high inorganic content in the seaweed that was not removed well by pre-washing. Therefore, KOH treatment actually did not decrease the sulfate level in final product of SRC but could have been involved in intra-molecular rearrangement to form more stable structure. Percentage of each parameter in KOH treated and untreated material is shown in Table 3. No soluble fiber was found in SRC obtained from KOH treatment, but it was 3.4% in untreated material. There was no statistically significant decrease in sulfate level up to 18% KOH treatment, but it was gradual. Decrease in sodium level could be due to the replacement of potassium ion. Potassium ion increment along with sulfate level might maintain electrical neutrality of the molecule. The rise in K^+ content in SRC samples shows that the charge neutrality of sulfate requires K^+ ion. It becomes evident that the amount of positively charged cations in the studied SRC preparations slightly exceeded the quantity needed for the complete neutralization of the negative charge of the polysaccharide backbone, revealing the presence of small amount of free salts (Rando, 2009). The increase in ash content could be due to increase in inorganic matter in the molecule, therefore, no sulfate removal occurred

Table 3. Material balance of raw seaweed and semi-refined carragenan prepared with different KOH concentrations

Composition	Raw seaweed (%)	Conc. of KOH used for preparation of SRC (%)				
		0	6	12	18	24
Moisture	33.14±2.19	8.55±0.92	9.44 ±0.62	10.8±0.66	10.91±1.07	10.75±1.11
Salt	27.30±0.17	2.15±0.21	0.34±0.00	0.42±0.00	0.40±0.00	0.38±0.01
Waste Solids	1.92±0.12	0.72±0.02	0.14±0.00	0.12±0.01	0.11±0.00	0.12±0.00
Soluble polymer	5.61±0.1.20	3.40±0.62	0	0	0	0
Fiber	7.75±1.32	20.85±2.05	17.44±1.14	16.98±1.72	16.12±1.55	16.90±0.89
Carrageenan	24.28±1.88	64.33±3.18	72.64±2.67	71.68±2.48	72.46±2.81	71.85±2.11
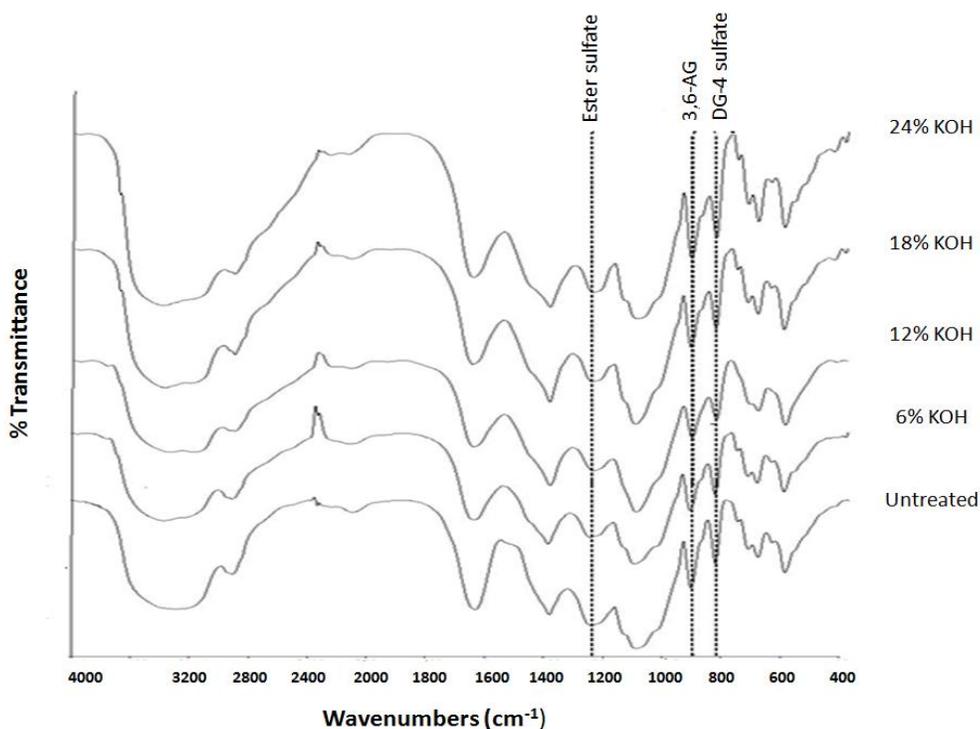
Figure 1. Fourier Transform Infrared Spectra of semi-refined prepared from *K. alvarezii* with different KOH concentrations.

even when cooked at higher KOH concentrations; rather, it showed higher level of sulfate which could be due to removal of alkali soluble protein and low molecular weight carbohydrates.

FTIR spectroscopy (Figure 1) shows that the molecules present in all four SRC samples are quite similar, as spectral band was at 1257 cm^{-1} which referred to ester sulfate of 930 cm^{-1} for 3,6-ag and 848 cm^{-1} assigned to galactose-4-sulfate (van de Velde et al., 2002; Pereira et al., 2009a). Wave length (cm^{-1}) ranging from 1220 to 1260cm^{-1} determines the presence of ester sulfate, since there is a linked bond of S-O in the ester sulfate. Wave lengths from 1010 to 1080 are assigned to glycosidic bond, while lengths from 928 to 930, 840 to 850, 825 to 830, 810 to 820 and 800 to 805 indicate the presence of 3,6-AG galactose-4-sulfate, galactose-2 sulfate, galactose-

6-sulfate and 3,6 anhydrogalactose-2-sulfate, respectively (Aguilan et al., 2003; Dewi et al., 2012). It was reported that during the process of seaweeds extraction, the methods do not induce any significant change in the molecular structure of the native seaweed final product (Dewi et al., 2012). Rando et al. (2006) observed only small changes in the sulfur concentration of hybrid carrageenans extracted by water or an alkali solution, which is negligible to be considered a desulfation reaction. The 3,6-AG content KOH untreated material was 28.30±0.52% and in SRC samples obtained from treating with KOH concentration of 6, 12, 18 and 24% were 33.41±0.50, 32.97±.42, 31.15±0.60 and 31.61 ±0.71%, respectively. Therefore, it was 18.06, 16.50, 10.07 and 10.70%, respectively, more than KOH untreated material. SRC prepared with low conc. of KOH

(6%) contained higher 3,6-AG (18.06%) and it is in agreement with observation made by Rando et al. (2006). The KOH untreated material did form gel in plain water, but it formed 97±10.27g cm^{-2} of KCl gel strength. KCl gel strength of SRC obtained from 6, 12, 18 and 24% KOH treatment was 570.10, 556.70, 469.87 and 442.27% respectively more than control (Table 2).

Olav et al. (1967) observed that the main effect of the alkali treatment is to transform the "intermediate" fraction into a fraction which precipitates at KCl concentrations below 0.125 M, that is, similar or identical to the χ-fraction. The authors reported that the main effect was on the "intermediate" fraction, leading to a marked increase of the 3.6-AG content and gel strength of this material. The difference between this fraction and the χ-fraction after the treatment is negligible compared to the differences between the untreated fractions. The inter-mediate fraction is, however, potentially a gel forming agent, in that an alkali treatment transforms it into material with approximately the same gel-forming ability of the χ-fraction from the same species (Olav et al., 1967).

Stanley (1963) investigated the carrageenan of Irish moss that monoester sulfate groups present in the carrageenan are of a highly resistant nature and have not succeeded in effecting any extensive removal of monoester sulfate groups from the carrageenan without severely de-polymerizing the polysaccharide portion of the molecule of the carrageenan. It is seemingly the case that any alkali can affect a loosening or detachment of the carbon-oxygen-sulfur bond attaching the monoester sulfate to the polysaccharide portion of the carrageenan molecule. But this reaction is completely reversible so that no extensive removal of the monoester sulfate groups can occur, except in the presence of a reagent such as barium hydroxide, which is capable of removing the liberated sulfuric acid from the reaction scene. It was used to describe the intra-molecular rearrangement that involves migration of sulfate from 6th carbon to some other adjacent position (Stanley, 1963). Similarly, Olav et al. (1967) reported that sulfate content of carrageenan extracted from *Chondrus crispus* (Linnaeus) J. Stackhouse, *Gigartina stellata* (Stackhouse) Batters and *Gigartina skottsbergii* Setchell & N.L. Gardner before and after treatment with 1 N KOH, 100°C for 1 h cooking did not change the sulfate content much. That is, it was 32.2, 33.7, 34.0% before treatment and after treatment it was 32.0, 33.1, and 33.5%, respectively, but there was increase in 3,6-AG content of 34, 31.5 and 43.5% for *C. cripsus, G. stellata* and *G. skottsbergii*, respectively. This also proves that there is no apparent correlation between sulfate level and gel strength. Gel strength also increases 3.5 folds, 10.5 folds and 14 folds respectively for *C. cripsus, G. stellata* and *G. skottsbergii*. In the case of *Furcellaria lumbricalis* (Turner) J.V. Lamouroux, sulfate level before and after alkali treatment was 18.6 and 18.4%, respectively, showing no desulfation; but gel

strength increased from 500 (untreated) to >1000 for treated weed (Olav et al., 1967). The composition of *K. alvarezii* galactan has been widely investigated. The hybrid polysaccharides from this species consist mainly of κ-carrageenan with small amounts of ι-structure. Also, minor quantities of μ-carrageenan, ν-carrageenan and G (3-linked β-D-galactopyronose), L (4-linked α-L-galacto-pyranose) and G6M,4S (3-linked 6-O-methyl-β-D-galacto-pyronose-4-sulfate) residues have been reported to be components of this polysaccharide (Estevez et al., 2000; 2004; Pereira et al., 2009b). The exact chemical nature, inorganic part composition and purity of the separated polysaccharides are strongly dependent on the isolation procedures. The sulfur content of the three main carrageenan types usually remains in the range of 7 to 10% for κ-carrageenan, 9.5 to 11.5% for ι-carrageenan and 11 to 13% for λ-carrageenan. Based on the idealized structure types, the theoretical (calculated) sulfur content values of the highly sulfated carrageenans appear to be somewhat higher: 8.3% for κ-carrageenan (Rando, 2009).

Extraction of carrageenans from *Eucheuma cottonii* resulted in 61% of polysaccharides during the 4 h extraction in pure water; slightly lower yields (57%) were obtained if 0.02 M KOH solution was used as the extraction medium. Somewhat, lower yields in the case of alkaline extraction media (compared to pure water) have also been reported for other *Eucheuma* species (Freile-Pelegrin et al., 2006). A slight increase in the AG content by 1.6% for furcellaran and by 2.1% for *C. truncatus* galactons during the alkaline extraction in 0.02 M KOH solution was observed (compared to the water extracted preparation), indicating the presence of alkali-labile precursor residues. In the same way AG content increases, slightly increases in case of *K. alvarezeii*. The ^{13}C-NMR spectrum of the polysaccharide from *E. cottonii* confirmed the presence of κ-carrageenan (G4S-DA) as major component, and also indicted the presence of minor amounts of DA2S, D6S, 6S and G6M residues (I) (Rando, 2009).

Higher alkali contents in extracting media resulted in products with substantially declined molecular weight (Mw) characteristics and impaired gelling abilities. Compared to water extracted furcellaran samples gelled in salt solutions, nearly two times higher gel strength values were observed for the preparations obtained by the extraction process involving alkali metal hydroxides. This is mainly caused by the lower Mw values of the water extracted galactans, the higher content of divalent cations (Mg^{2+}, Ca^{2+}) preserving their composition and the effect of chloride ions. Compared to pure distilled water, the low yield on extraction using KOH may be due topolymer destruction. Polymer destruction produced the low molecular weight material which cannot be precipitated using alcohol (Rando, 2009). From this point of view, it can be described that sulfate removal is not a significant step and decrease in the alkali soluble matter

from the raw material increases the sulfate content indirectly. Iain (2008) reported that 15% sulfate in raw *Eucheuma* material increased to 16.6% in final product of SRC after reduction of 10% alkali soluble matter while cooking. This proves the increase in Ester sulfate content after SRC conversion, hence no significant desulfation. Therefore, the purpose of alkali treatment in the process of SRC/Carrageenan is to increase the stability of carrageenan polymer through molecular rearrangement and remove low molecular weight compounds from the seaweed.

Therefore, alkali treatment could be more of molecular reorientation process as to form a stable structure than meager sulfate removal in the process of SRC from *K. alvarezii*. From the present study, it can be concluded that the increased level of sulfate in the final SRC product could be due to removal of protein and cold water soluble low molecular weight compounds during processing. From the present investigation, it was observed that sulfate removal and quality improvement with increasing concentration of KOH for cooking seaweed is not statistically significant; therefore, seaweed can be subjected to lower concentration of KOH treatment (6 to 12%) to produce semi-refined carrageenan on commercial scale.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Aguilan JT, Broom JE, Hemmingson JA, Dayrit FM, Montano MNE, Dancel MCA, Ninonuevo MR, Furneaux RH (2003). Structural analysis of Carrageenan from farmed varieties of Philippines seaweed. Botanica Marina 46:179-192.
Campo VL, Kawano DF, Silva Junior DB, Ivone C (2009). Carrageenans: biological properties, chemical modifications and structural analysis. Carbohydr. Polym. 77:167-180.
Christopher SR, Michael GB (1998). Method for extracting semi-refined carrageenan from seaweed. US patent No. 5,801,240.
Cruchaga S, Echeita SA, Aladuena A, Garciapena J, Frias N, Usera MA (2001). Antimicrobial resistance in salmonella from human, food and animals in spain in 1998. Antimicrob. Chemother. 47:315-21.
Dewi EN, Darmonto YS, Ambariyanto (2012). Characterization and quality of semi refined carrageenan (SCR) products from different coastal waters based on fourier transform infrared technique. J.

Coast. Dev. 16(1):25-31.
Estevez JM, Ciancia M, Cerezo AS (2000). The system of low-molecular-weight carrageenans and agaroids from the room-temperature-extracted fraction of *Kappaphycus alvarezii*. Carbohydr. Res. 325(4):287-299.
Estevez JM, Marina C, Alberto SC (2004). The system of galactans of the red seaweed, *Kappaphycus alvarezii*, with emphasis on its minor constituents. Carbohydr. Res. 339:2575-2592.
Freile-Pelegrin Y, Robledo D, Azamar JA (2006). Carrageenan of *Eucheuma isiforme* (Solieriaceae, Rhodophyta) from Yucatan, Mexico.I. Effect of extraction conditions. Bot. Marina 49:65-71.
Glicksman M (1983). Red Seaweed extracts (agar, carrageenans, furcellaran). In, Vol.2. M. Glicksman (ed.). Food hydrocolloids. Boca Raton: CRC Press, pp. 73-113.
Hoffmann R, Gidley M, Cooke D, Frith W (1995). Effect of isolation procedures on the molecular composition and physical properties *eucheuma cottonii* carrageenan. Food Hydrocoll. 9(4): 281-289.
Iain CN (2008). Basic manufacturing practices for raw-dried seaweed and semi-refined carrageenan from *Eucheuma* and *Kappaphycus*. SEAPlant.net Monograph no. HB2G 1008 V2 BMP
Jarvis B, Lach VH, Wood JM (1977). Evaluation of the spiral plate maker for the enumeration of microorganisms in foods. J. Appl. Bacteriol. 43:149-157.
JECFA (2010). Processed *Eucheuma cottonii*, www.marinealg.org, assessed on 9 January, 2015
Krishnan M, Narayanan R (2010). Structure, conduct and performance of value chain seaweed farming in India. Agric. Econ. Res. Rev. 23:505-514.
McHugh DJ (2003). A guide to the Seaweed industry. FAO Fisheries technical paper 441. Rome: FAO. pp. 61-72.
Mehta AS, Mody KH, Anita I, Ghosh PK (2008). Preparation of semi-refined k-carrageenan: Recycling of alkali solution and recovery of alkali from spent liquor. Ind. J. Chem. Tech 15:45-52.
Olav S, Bjorn L, Alberto JP, Arne H (1967). The effect of alkali treatment on the chemical heterogeneity and physical properties of some carrageenans. Acta Chem. Scand. 21:2585-2598.
Pereira L, Amado AM, van de Velde F, Ribeiro-Claro PJA (2009a). Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). Food Hydrocoll. 23(7):1903-1909
Pereira L, Critchley AT, Amado AM, Ribeiro-Claro PJA (2009b). A comparative analysis of phycocolloids produced by underutilized versus industrially utilized carrageenophytes (Gigartinales, Rhodophyta). J. Appl. Phycol. 21(5):599-605
Philips GO (2002). The chemical identification of PNG carrageenan. Newtech Innovation Centre, Croesnewydd Hall, Wrexham LL13, 7YP, Wales, UK.
Rando T (2009). Functional dependencies of the chemical composition and structures in the Baltic sea algal communities. Ph.D. Thesis, online, PDF. Assessed on 24 January 2014.
Rando T, Kalle T, Merike V, Tiiu K, George M, Priit K (2006). The extraction and quantification of hybrid carrageenan from the biomass of the red algae *Fucellaria lumricalis* and *Coccotylus truncatus*. Proc. Estonian Acad. Sc. Chem. 55(1):40-53
Sperisa D, Wiratni, Fahururrozi M, Rochmadi (2011). Carrageenan properties extracted from *Eucheuma cottonii*, Indonesia. World Acad. Sci. Eng. Technol. 54:738-742.
Stanley NF (1963). Process for treating a polysaccharide of seaweeds of the Gigartinaceae and Solieriaceae families. US Patent, patent no. 3094517.
van de Velde F, Knusten SH, Usov AI, Romella HS, Cerezo AS (2002). ¹H and ¹³C high resolution NMR spectroscopy of Carrageenans: application in research and industry. Trends Food Sci. Technol. 13:73-92.
Yaphe W, Arsenault GP (1965). Improved resorcinol reagent for the determination of fructose and 3,6-anhydrogalactose in polysaccharide. Anal. Biochem. 13:143-148.