Botanica Marina 49 (2006): 65–71 © 2006 by Walter de Gruyter • Berlin • New York. DOI 10.1515/BOT.2006.008

Carrageenan of *Eucheuma isiforme* (Solieriaceae, Rhodophyta) from Yucatán, Mexico. I. Effect of extraction conditions

Yolanda Freile-Pelegrín[1,*], Daniel Robledo[1] and José A. Azamar[2]

[1] Department of Marine Resources, CINVESTAV, Km 6 Carretera Antigua a Progreso, Cordemex, 97310, A.P. 73, Mérida, Yucatán, Mexico, e-mail: freile@mda.cinvestav.mx

[2] Department of Applied Physics, CINVESTAV, Km 6 Carretera Antigua a Progreso, Cordemex, 97310, A.P. 73, Mérida, Yucatán, Mexico

* Corresponding author

Abstract

The yield and physicochemical properties of carrageenan from *Eucheuma isiforme* harvested at the Yucatán coast were investigated. Carrageenan was extracted under different alkali concentrations (0, 1, 3, 5 and 7% KOH) and treatment durations (3, 4 and 5 h). Native carrageenan, extracted without KOH, had the highest yield (~44.6%) independently of treatment duration. After alkali treatment, carrageenan yield ranged from 35.3 to 31.8%. No significant differences in carrageenan yield were observed between 1 and 3% KOH. Native carrageenan had low viscosity values (39–57.0 cPs), whereas carrageenan extracted with 1% KOH at 3 and 4 h increased in viscosity (160.0–161.3 cPs). Alkali-treated carrageenan formed very weak gels (<50 g cm^{-2}) in 1.5% solutions. The chemical analysis and FTIR spectra revealed a preponderantly iota-carrageenan extract. Extractions performed with 1% KOH for 3 h produced carrageenan with suitable properties to be considered as a substitute for traditional iota-carrageenan sources.

Keywords: carrageenan; *Eucheuma isiforme*; extraction; gel properties; structure.

Introduction

Carrageenans are sulfated galactans composed of D-galactose residues linked alternately in α-1,3 and β-1,4 bonds. They are classified as kappa, iota or lambda according to their sulfate substitution pattern and 3,6-anhydrogalactose content. Worldwide use of carrageenan in food applications has been growing 5–7% per annum, with primary application in the dairy industry for stabilizing and texturing products, particularly flavored milk (Bixler 1996). If this trend continues, more than25 000 metric tons of carrageenan will be needed by 2005 to meet industry needs. Current carrageenan sources are insufficient to meet demand, particularly given the processing industry's quality, price and volume flow requirements (Ask et al. 2003). For the last thirty years seaweed supply for carrageenan production has been restricted largely to harvesting of natural *Chondrus crispus* Stackhouse in Canada and France. However, carrageenan production is now dominated by the warm water species *Kappaphycus alvarezii* Doty and *Eucheuma denticulatum* (Burnan) Collin *et* Harvey, cultivated mainly in the Philippines and Indonesia (McHugh 2001).

In Mexico, temperate carrageenophytes from the Pacific coast have been analyzed for carrageenan content (Correa-Díaz et al. 1990) while the Gulf of Mexico native *Eucheuma isiforme* (C. Agardh) J. Agardh has been partially studied. This species was harvested and exported to Denmark during the late 1970s for iota carrageenan production during a supply shortage of Philippine *E. denticulatum*, the main source for iota-carrageenan (Peter Salling pers. comm.). Mariculture of *E. isiforme* on the Yucatán Peninsula has been proposed because the regional environmental and oceanographic conditions are suitable for tropical seaweed farming (Perez-Enriquez 1996, Muñoz et al. 2004) and its potential use in the carrageenan industry (Robledo 2005). Thus, between 150 and 400 tons of carrageenan were imported to Mexico in the 1990s, but current domestic demand has increased to approximately 2600–3000 tons (Robledo 2005). To date, there is only one company producing carrageenan in Mexico, though its production relies on carrageenophytes imported from Asia.

Several factors are known to affect carrageenan yield and quality, including: the algal species (Craigie 1990), seasonal fluctuations (Dawes et al. 1977, Yakovleva et al. 2001) and extraction conditions (Dawes et al. 1977, McCandless et al. 1977, Hoffmann et al. 1995, Piculell 1995). The natural precursors of kappa- and iota-carrageenan (mu and nu, respectively) are non-gelling carrageenans due to irregularities in the 6-sulfate ester groups on some D-galactose. Most of these 6-sulfate units convert to the corresponding 3,6-anhydro-D-galactose during alkaline industrial carrageenan extraction, imparting a higher degree of regularity to the molecule.

Long reaction times and highly alkaline conditions are used commercially to achieve a high level of precursor conversion to kappa- and iota-carrageenan when maximizing gelling ability and/or protein reactivity in foods (Falshaw et al. 2001). The present study evaluated the quality of carrageenan from the tropical seaweed *Eucheuma isiforme* in preparation for its commercial production in Yucatán. Different alkaline conditions and

treatment duration were used to extract carrageenan. Native and alkali-treated carrageenan properties (i.e., yield, gel strength and viscosity) are described. Structural analyses were also done, including sulfate, 3,6-anhydro-D-galactose content and infrared spectroscopy.

Materials and methods

Sample collection and carrageenan extraction

Eucheuma isiforme was collected manually from natural beds at Dzilam de Bravo (21°03′00″ N, 88°57′50″ W), Yucatán, Mexico, in May 1998. Thalli were washed thoroughly with tap water to remove excess salts and sand, oven-dried at 60°C and then milled prior to carrageenan extraction. The collected algal material was free of epiphytes and thus considered "pure seaweed".

The carrageenan from *Eucheuma isiforme* was obtained using the hot alkaline extraction method described by Stanley (1987), with some modifications. Alkali concentrations between 0 and 7% KOH and treatment duration from 3 to 5 h were used. Dry samples (5 g) were rehydrated at room temperature for 12 h in the corresponding KOH solution (0, 1, 3, 5 and 7%), followed by the hot alkali extraction at 85°C at one of three treatment durations (3, 4 or 5 h). The extract was mixed with diatomaceous earth (Celite), pressure filtered and the filtrate neutralized to pH 8.9 with 5 M HCl prior to the recovery of the carrageenan from the solution.

Carrageenan was precipitated by slowly adding 250 ml of 2% Cetavlon (hexadecyl-tri-methylammonium bromide) in 9:1 (v/v) distilled water:acetone according to Craigie and Leigh (1978) and Chopin et al. (1990), and recovered over filter paper *in vacuo*. To remove Cetavlon residues, the carrageenan fibers were carefully washed three times with 63 ml of 95% ethanol saturated with sodium acetate. Sodium acetate residues were then removed by three final washes with 95% ethanol. Carrageenan was dried at 60°C for 24 h, weighed to calculate the percent yield, and milled. Extractions and carrageenan analyses were performed in triplicate for each treatment.

Rheological and chemical analysis

Rheological properties were measured for the carrageenans extracted at different alkali concentrations and treatment duration. Water gel strength was determined according to Freile-Pelegrín and Robledo (1997) in a 1.5% w/v carrageenan solution using a Nikansui Shiki gelometer with 1 cm^2 plunger (Kiya Seisakusho, Tokyo, Japan). Viscosity was measured using a Cole Parmer Viscosimeter (Vernon Hills, USA) with a low centipoise adapter at 20 rpm (spindle number 8) on 18 ml samples of a 1.5% carrageenan solution. Samples were homogenized and allowed to stabilize in a recirculating bath at 75°C.

Sulfate content was measured turbidimetrically after hydrolyzing 25 mg carrageenan in sealed tubes for 12 h in 1N HCl at 105°C (Jackson and McCandless 1978). The 3,6 anhydrogalactose content (3,6 AG) was determined following Matsuhiro and Zanlungo (1983) and total carbohydrates were obtained by the phenol sulfuric acid method (Dubois et al. 1956). These values were used to calculate the molar ratio of galactose to 3,6 AG to ester sulfate. The galactose content is expressed as total carbohydrate content in the algae minus the corresponding 3,6 AG content for each extraction condition.

Carrageenans extracted at different alkali and treatment duration treatments were analyzed by Fourier transformed infrared spectroscopy (FTIR). About 4.0 mg of carrageenan were mixed thoroughly in a mortar with 200 mg of potassium bromide until homogenized. The infrared spectra of native and alkali-modified carrageenan were recorded on a ThermoNicolet Nexus 670 FTIR spectrometer (Madison, WI, USA) equipped with a DTGS KBr detector and purge gas generator at a spectral resolution of 0.09 cm^{-1} and a wave length precision of 0.01 cm^{-1}. Each spectrum (32 scans) was acquired at a resolution of 4 cm^{-1}.

Statistical analysis

Data were tested for normality (Kolmogorov-Smirnov) and homogeneity of group variances (Bartlett's test) using statistical software (Statistica 6.0, Statsoft). When necessary, variables were log x or log (x+1) transformed to meet these assumptions. The interactions of KOH concentration and treatment duration with carrageenan properties were assessed with a two-way analysis of variance (ANOVA). A *post hoc* analysis of the means using the Tukey (HSD) test was done to compare between treatments, and a Pearson's correlation coefficient was used to determine the correlation between carrageenan properties.

Results

Carrageenan content and properties

Eucheuma isiforme extracted without KOH (native carrageenan) had the highest yields, with 44.5% at 3 h, 44.1% at 4 h and 45.3% at 5 h. No significant differences between treatment durations at 3 and 4 h were found ($F_{1,4}$=1.71, $p>0.05$) (Figure 1). Carrageenan yield decreased after alkali treatment using 1 and 3% KOH, with values ranging from 31.8 to 35.3%. Yield increased after treatment with 5 and 7% KOH, with a maximum value of 43.2% at 7% KOH for 5 h.

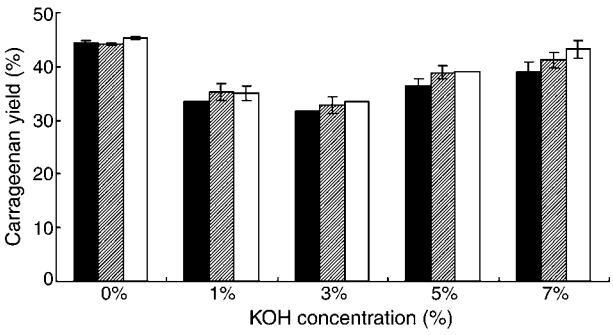

Figure 1 *Eucheuma isiforme*: carrageenan yields of native and alkali treated carrageenans obtained at different treatment durations: 3 h (white bar), 4 h (cross-hatched bar) and 5 h (black bar). Means±SD; n=3.

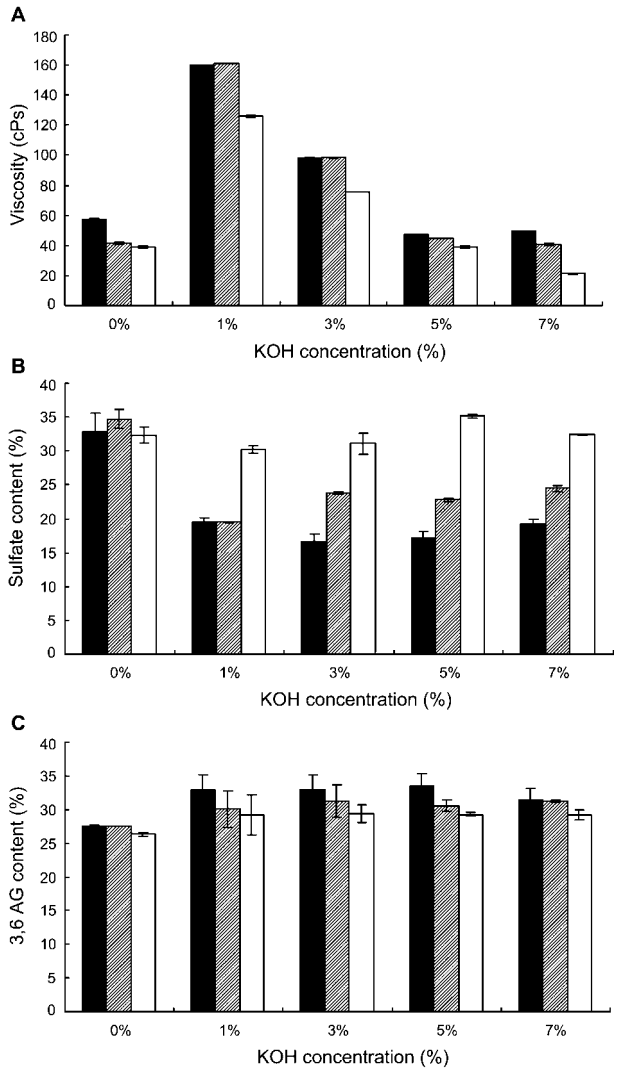

Figure 2 *Eucheuma isiforme*: carragenan properties of native and alkali treated carrageenans obtained at different treatment durations: 3 h (white bar), 4 h (cross-hatched bar) and 5 h (black bar).
(A) Viscosity, (B) sulfate content, (C) 3,6 AG content. Means±SD; n=3.

Carrageenan viscosities are shown in Figure 2A. Native carrageenan showed significant differences ($F_{2,6}$=347.9, $p<0.01$) in viscosities between treatment durations at 3 h (57.0±0.9 cPs), 4 h (41.6±0.9 cPs) and 5 h (39.0±0.9 cPs). The 1% KOH treatment resulted in a two-fold increase in viscosity in comparison to native carrageenan. Carrageenan viscosity at 3 h was 160.0 cPs and for 4 h treatment duration it was 161.3 cPs, whereas at 5 h a 21.9% reduction in viscosity was observed. At 3% KOH, viscosities decreased by 38.8% at 3–4 h and by 53.1% at 5 h. Extraction at 5 and 7% KOH produced carrageenan with viscosities below 50 cPs. An inverse relationship between carrageenan yield and viscosity was observed for all treatments (r=-0.63 for 3 h; r=-0.79 for 4 h; r=-0.79 for 5 h; $p<0.01$). Alkali-treated carrageenan formed soft gels (<50 g cm^{-2}) while native carrageenan did not gel at all.

Carrageenan sulfate content is shown in Figure 2B. Native carrageenan sulfate content varied from 32.3 to 34.7% with no significant differences between treatment durations ($F_{2,5}$=1.68, $p>0.05$). In general, alkali treatment reduced carrageenan sulfate content by ~40% at 3 h and ~22% at 4 h, with values ranging from 16.6 to 24.5%. No significant decrease in sulfate content was observed in carrageenan extracted with alkali for 5 h in comparison to native carrageenan ($p>0.05$).

Carrageenan 3,6 AG content is shown in Figure 2C. The lowest 3,6 AG contents (26.3–27.47%) were recorded for the native carrageenan, with no significant difference between treatment durations at 3 and 4 h ($F_{1,4}$= 0.013, $p>0.05$). An increase of 14% was observed after alkali treatment (contents ranging from 29.2 to 33.0%). The highest 3,6 AG contents were recorded in carrageenan extracted at 3 h and decreased as treatment duration increased (4–5 h), independently of KOH concentration. A negative correlation was noted between carrageenan yield and 3,6 AG content (r=-0.72 for 3 h, $p<0.01$; r= -0.51 for 4 h, $p<0.05$; r=-0.53 for 5 h, $p<0.05$). A clear inverse relationship was observed between 3,6 AG and sulfate contents at 3 h (r=-0.68, $p<0.01$) and 4 h (r= -0.55, $p<0.05$), but not at 5 h.

The individual and combined effects of KOH concentration and treatment duration on carrageenan properties are shown in Table 1. Alkali concentration and treatment duration had a significant interaction effect on viscosity and sulfate content.

Molar ratios

Molar ratios of galactose to 3,6 AG to ester sulfate were calculated based on the total carbohydrate content in the algae (54.5%) (Table 2). Native carrageenan molar ratios were similar across treatment durations. Molar ratios varied after alkali treatment with an increase in 3,6 AG and a decrease in sulfate. This pattern was more evident for 3 h than 4 h treatment duration, across all KOH concentrations. It is noteworthy that for 5 h treatment duration molar ratios were similar to those obtained for native carrageenan.

FTIR spectroscopy

The FTIR spectra for the native and alkali-treated carrageenan from *Eucheuma isiforme* are shown in Figure 3. All spectra displayed an absorption band at 1220–1240 cm^{-1} related to sulfation level (Stancioff and Stanley 1969). A particularly intense signal was recorded in all samples at 803–805 cm^{-1}, which is specific to 3,6-anhydrogalactose-2-sulfate. Another signal was observed at 845–847 cm^{-1} (attributed to galactose-4-sulfate). Peaks were also observed at 965–975 cm^{-1} in all samples (3-linked β-D-galactose 4-sulfate and 4-linked α-D-galactose 2-sulfate). In general, the carrageenan from *E. isiforme* extracted at 5 h had the highest intensities at this signal (peak). The intense signal observed around 930 cm^{-1} was consistent with the presence of 3,6 AG (Stancioff and Stanley 1969). A slight intensity increase at this peak was evident between the native and alkali-treated carrageenans implying either absence or undetectable levels of the precursor, 1,4-linked galactose-6-sulfate.

The ratio between 805 and 845 cm^{-1} absorption bands in FTIR spectra was calculated (Rochas et al. 1986,

Table 1 *Eucheuma isiforme*: two-way analysis of variance for carrageenan yield, viscosity, sulfate content and 3,6 AG.

Variable	Sums of squares	Degrees of freedom	F-value	p-value
Yield				
A	211.90	4	92.23	<0.001*
B	18.58	2	0.86	ns
A×B	1.95	8	1.49	ns
Viscosity				
A	0.59	4	78.22	<0.001*
B	0.10	2	1.73	ns
A×B	0.01	8	386.63	<0.001*
Sulfate content				
A	0.04	4	4.49	0.005*
B	0.12	2	22.93	<0.001*
A×B	0.01	8	43.39	<0.001*
3,6 AG content				
A	0.00	4	7.17	0.002*
B	0.00	2	6.34	0.004*
A×B	0.00	8	0.57	ns

A=KOH concentration; B=treatment duration; A×B=KOH concentration and treatment duration interaction.
* Highly significant ($p<0.001$); ns=not significant ($p>0.05$).

Correa-Díaz et al. 1990, Pereira and Mesquita 2003) and used as a qualitative parameter to determine the degree of iota/kappa hybridization of the native and alkali-treated samples. The ratio for native carrageenan extracted at 3 h and 4 h treatment duration was 0.65. Native carrageenan extracted at 5 h had a ratio similar to those obtained for all alkali treated carrageenan (1.00).

Discussion

The native carrageenan yields of *Eucheuma isiforme* from Yucatán were similar to those obtained by Dawes et al. (1977) from Florida material. In the present study, yield decreased as alkali concentration increased, which may be related to degradation of the polysaccharide resulting from the alkali concentration used. Dawes et al. (1977) reported higher carrageenan yields after alkali transformation (59.7%) than in the present results, though Perez-Enríquez (1996) obtained similar yields from *E. isiforme* from Yucatán extracted with the same method described in Dawes et al. (1997).

Carrageenan viscosity increased after alkali modification. However, a gradual decrease in this parameter was observed. These viscosity values are in the ranges of those reported for *Eucheuma isiforme* from Florida (Dawes et al. 1977) and for other iota-producing species (Azanza-Corrales and Sa-a 1990, Brenden and Bird 1994). The KOH concentrations above 1% used in the present study may have caused carrageenan degradation, with an evident decrease in viscosity. The hot alkaline extraction operations inevitably involve some degradation of the polysaccharide due to the rigors (heat, alkalinity) of processing (Stanley 1987). Although carrageenans are reasonably stable under alkaline conditions, severe conditions, such as KOH concentrations >3% and extended treatment duration could promote depolymerisation of the carrageenan (Critchley, pers. comm.). Similar results were found by Dawes et al. (1977) for *E. isiforme* from Florida, where high lime concentrations and duration produced a drop in carrageenan gel strength and viscosity.

Table 2 *Eucheuma isiforme*: molar ratios of sugar residues and sulfate content found in carrageenan extracted under the experimental conditions.

KOH (%)	Molar ratio of galactose:3,6 AG:sulfate		
	3 h	4 h	5 h
0	1:0.73:2.27	1:0.73:2.40	1:0.73:2.20
1	1:1.00:1.67	1:0.92:1.53	1:0.78:2.21
3	1:1.00:1.54	1:0.92:1.92	1:0.80:2.28
5	1:1.00:1.54	1:0.92:1.84	1:0.80:2.64
7	1:1.00:1.48	1:0.92:1.92	1:0.80:2.41

Native carrageenan sulfate contents are within the range for iota-producing *Eucheuma* species (Cheney et al. 1987, Santos 1989, Fostier et al. 1992). Both alkali concentration and treatment duration significantly affected sulfate content (Table 1), with maximum sulfate reduction occurring at 3 h for all the alkali concentrations tested (Figure 2B). The 3,6 AG contents in alkali-treated carrageenan from *Eucheuma isiforme* were higher than previously reported for this species (26%) and for *E. spinosum* J. Ag. (nom. illeg.) (25.3%) by Fostier et al. (1992). In contrast, Dawes et al. (1977) reported lower values for Floridian *E. isiforme* (19.4%). The present data for 3,6 AG contents in carrageenan from *E. isiforme* were also higher than those reported for other iota-producing seaweeds precipitated with Cetavlon [i.e., 15.7% in *Agardhiella subulata* (C. Agardh) Kraft *et* Wynne, Chopin et al. 1990; and 14.5% in *Cryptonemia crenulata* (J. Agardh) J. Agardh (Saito and de Oliveira 1990)]. The KOH concentration and treatment duration significantly affected sulfate content and 3,6 AG content in the carrageenan extracted (Table 1). An inverse correlation between sulfate and 3,6 AG was observed, except at 5 h treatment duration. Under these conditions, there is no evidence for a transformation of the precursor since neither sulfate reduction nor increase in 3,6 AG occurs. Probably, severe extraction conditions, such as strong alkali concentration and extended treatment duration, affect carrageenan.

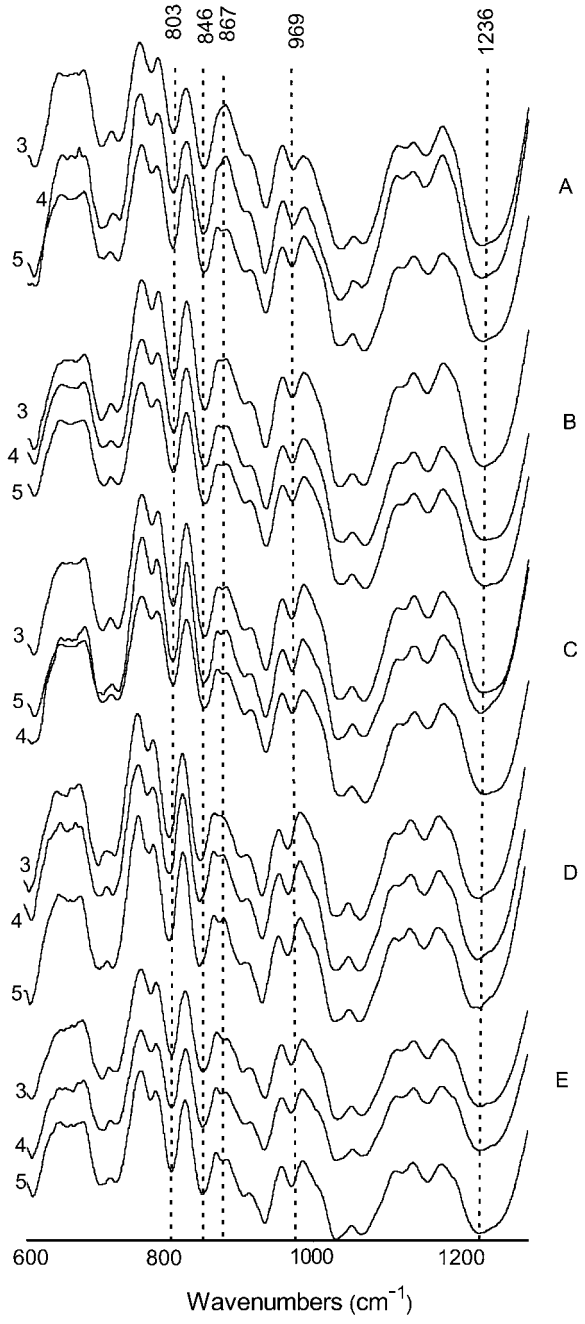

Figure 3 *Eucheuma isiforme*: FTIR spectra of native and alkali treated carrageenans obtained at 3, 4 and 5 h.
(A) 0% KOH, (B) 1% KOH, (C) 3% KOH, (D) 5% KOH, (E) 7% KOH.

Nevertheless, other analytical techniques may be used to corroborate this.

The native carrageenan from *Eucheuma isiforme* from Yucatán did not gel, which agrees with previous reports for the species from Florida (Dawes et al. 1977). With alkali conversion, 3,6 AG content promotes double helix formation and thus gelation. Very weak carrageenan gels were produced in *E. isiforme* from Yucatán after alkali treatment (<50 g cm^{-2}). Similar values were reported by Santos (1989) for the same species (53 g cm^{-2}), while higher values were found by Dawes et al. (1977) for modified carrageenan (318 g cm^{-2}). However, comparison between these two studies is difficult because the former reported gel strength for carrageenan gels 2% w/v with potassium chloride (0.2).

The FTIR spectra showed an intense signal at 803–805 cm^{-1}, which is specific of iota carrageenan (Dawes et al. 1977). However, the signal at 845–847 cm^{-1} corroborates the existence of a kappa- and iota-carrageenan mixture (Chopin et al. 1990). All spectra exhibited a shoulder at 867 cm^{-1}, attributed to the sulfate group at C-6, indicating the presence of nu-carrageenan, considered the biological precursor to iota-carrageenan (Bodeau-Bellion 1983). This was corroborated through chemical analysis that showed an increase in 3,6 AG content and a reduction in sulfate after alkali modification at 3 h and 4 h treatment duration. The biological precursors of kappa and iota carrageenan (mu and nu, respectively) both contain a sulfate ester group at position C-6 of a 4-linked α-D-galactose unit with characteristic bands at 820 and 867 cm^{-1}, respectively (Bodeau-Bellion 1983, Chopin et al. 1990, Pereira and Mesquite 2003). In *Eucheuma isiforme* from Yucatán, nu-carrageenan may, therefore, be the common precursor for both kappa- and iota-carrageenan since the predicted precursor of kappa-carrageenan (D-galactose-6-sulfate) was not detected (peak at 820 cm^{-1}). Bellion et al. (1981) described this for the kappa-, iota- and nu-carrageenan family. The hybrid structure of kappa-, iota- and nu-carrageenan has also been reported in *E. isiforme* var. *denudatum* Cheney [as *E. nudum* (Greer and Yaphe 1984)] and in *Agardhiella subulata* (Chopin et al. 1990). The peak at 867 cm^{-1} was not recorded in previous studies of carrageenan from *E. isiforme* (Lawson et al. 1973, Dawes et al. 1977). As Chopin et al. (1990) pointed out, the presence of a low abundance carrageenan component cannot be detected by IR spectroscopy, though FTIR spectroscopy is a much more powerful tool.

The FTIR spectra and molar ratios indicated that the phycocolloids extracted from *Eucheuma isiforme* from Yucatán have a dominant iota-carrageenan which is similar to *E. spinosum*, a current commercial source of iota-carrageenan (Deslandes et al. 1985). Theoretically, the molar ratio of galactose to 3,6 AG to ester sulfate for iota carrageenan is 1:1:2 (Anderson et al. 1973). In *E. isiforme* from Yucatán the galactose to 3,6 AG ratios increased after alkali modification, except for 5 h treatment duration. This pattern has been described by Lawson et al. (1973) and Dawes et al. (1977) for the same species, though *E. isiforme* from Yucatán had higher 3,6 AG contents in both the native carrageenan (1:0.73) and alkali-treated carrageenan (1:1) (Dawes et al. 1977, 1:0.4).

In conclusion, *Eucheuma isiforme* from Yucatán may be a good source of relatively pure iota-carrageenan if extracted under certain conditions. Extraction with 1% KOH at 3 h produces carrageenan of sufficient quality to serve as a substitute for traditional carrageenan sources since sulfate levels fit the US Food and Drug Administration purity standard of 20–40% (dry weight) and meet the industrial requirements of minimum carrageenan yield and viscosity values of 39% (dry weight) and 5 cPs, respectively (Bixler 1996). Higher KOH concentrations at 3 h and 4 h notably decrease viscosity, suggesting that alkali concentrations <1% should be investigated. Future

research should focus on use of *E. isiforme* carrageenan for industrial applications, as well as more detailed characterizations of carrageenan composition, perhaps through NMR spectroscopy.

Acknowledgements

We thank C. Chávez Quintal for her technical assistance during laboratory analysis. This study was supported by the projects CONACYT-SAGARPA 2002-C01-1057 and CONACYT 38490E.

References

Anderson, N.S., T.C.S. Dolan and D.A. Rees. 1973. Carrageenans. Part VII. Polysaccharides from *Eucheuma spinosum* and *Eucheuma cottonii*. The covalent structure of ι-carrageenan. *J. Chem. Soc. Perkin Trans. 1*: 2173–2176.

Ask, E., A. Batibasaga, J.M. Zertuche-González and M. de San. 2003. Three decades of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic location. *Proc. Int. Seaweed Symp. 17*: 49–57.

Azanza-Corrales, R. and P. Sa-a. 1990. The farmed *Eucheuma* species (Gigartinales, Rhodophyta) in Danajon Reef, Philippines: carrageenan properties. *Hydrobiologia 204/205*: 521–525.

Bellion, C., G.K. Hamer and W. Yaphe. 1981. Analysis of kappa-iota hybrid carrageenans with kappa-carrageenase, iota-carrageenase and 13C-NMR. *Proc. Int. Seaweed Symp. 10*: 379–384.

Bixler, H.J. 1996. Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia 326/327*: 35–57.

Bodeau-Bellion, C. 1983. Analysis of carrageenan structure. *Physiol. Vég*. *21*: 785–793.

Brenden, P.Ch. and K.T. Bird. 1994. Effects of environmental factors on carrageenan from *Gymnogongrus griffithsiae* (Gigartinales, Rhodophyta). *J. Appl. Phycol. 6:* 371–380.

Cheney, D.P., A.H. Luistro and P.M. Bradley. 1987. Carrageenan analysis of tissue cultures and whole plants of *Agardhiella subulata*. *Hydrobiologia 151/152*: 161–166.

Chopin, T., M.D. Hanisak, F.E. Koehn, J. Mollion and S. Moreau. 1990. Studies on carrageenan and effects of seawater phosphorus concentration on carrageenan content and growth of *Agardhiella subulata* (C. Agardh) Kraft and Wynne (Rhodophyceae, Solieriaceae). *J. Appl. Phycol. 2*: 3–16.

Correa-Díaz, F., R. Aguilar-Rosas and L.E. Aguilar-Rosas. 1990. Infrared analysis of eleven carrageenophytes from Baja California, Mexico. *Hydrobiologia 204/205*: 609–614.

Craigie, J.S. 1990. Cell walls. *In*: (K.M. Cole and R.G. Sheath, eds) *Biology of the red algae*. Cambridge University Press, Cambridge. pp. 221–258.

Craigie, J.S. and C. Leigh. 1978. Carrageenan and agar. *In*: (J.A. Hellebust and J.A. Craigie, eds) *Handbook of phycological methods: physiological and biochemical methods*. Cambridge University Press, Cambridge. pp. 109–131.

Dawes, C.J., N.F. Stanley and D.J. Stancioff. 1977. Seasonal and reproductive aspects of plant chemistry and iota carrageenan from Floridian *Eucheuma* (Rhodophyta, Gigartinales). *Bot. Mar. 20*: 137–147.

Deslandes, E., J.Y. Floc'h, C. Bodeau-Bellion, D. Brault and J.P. Braud. 1985. Evidence for ι-carrageenans in *Solieria chordalis* (Solieriaceae) and *Calliblepharis jubata*, *Calliblepharis ciliate*, *Cystoclonium purpureum* (Rhodophyllidaceae). *Bot. Mar. 28*: 317–318.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Roberts and F. Smith. 1956. Colorimetric method for determination of sugar and related substances. *Anal. Chem. 28*: 350–356.

Falshaw, R., H.J. Bixler and K. Johndro. 2001. Structure performance of commercial kappa-2 carrageenan. I. Structure analysis. *Food Hydrocolloids 15*: 441–452.

Fostier, A.H., J.M. Kornprobst and G. Combaut. 1992. Chemical composition and rheological properties of carrageenans from two Senegalese Solieriaceae *Anatheca montagnei* Schmitz and *Meristotheca senegalensis* Feldmann. *Bot. Mar. 35*: 351–355.

Freile-Pelegrín, Y. and D. Robledo. 1997. Effects of season on the agar content and chemical characteristics *of Gracilaria cornea* from Yucatán, Mexico. *Bot. Mar. 40*: 285–290.

Greer, C.W. and W. Yaphe. 1984. Enzymatic analysis of carrageenans: structure of carrageenan from *Eucheuma nudum*. *Hydrobiologia 116/117*: 563–567.

Hoffmann, R.A., D. Gidley, D. Cooke and W.J. Frith. 1995. Effect of isolation procedures on the molecular composition and physical properties of *Eucheuma cottonii* carrageenan. *Food Hydrocolloids 9*: 281–289.

Jackson, S.G. and E.L. McCandless. 1978. Simple, rapid, turbidimetric determination of inorganic sulfate and/or protein. *Analyt. Bio. 90:* 802–808.

Lawson, C.J., D.A. Rees, D.J. Stancioff and N.F. Stanley. 1973. Carrageenans. Part VIII. Repeating structures of galactan sulfates from *Furcellaria fastigiata, Gigartina canaliculata, Gigartina chamissoi, Gigartina atropurpurea, Ahnfeltia durvillaei, Gymnogongrus furcellatus, Eucheuma cottonii, Eucheuma spinosum, Eucheuma isiforme, Eucheuma uncinatum, Aghardhiella tenera, Pachymenia hymantophora*, and *Gloiopeltis cervicornis*. *J. Chem. Soc. Perkin I*: 2177–2182.

Matsuhiro, B. and A. Zanlungo. 1983. Colorimetric determination of 3,6-anhydrogalactose in polysaccharides from red seaweeds. *Carbohydr. Res. 118*: 276–279.

McCandless, E., W.T. Okada, J.N.A. Lott, C.M. Vollmer and E.M. Gordon-Mills. 1977. Structural studies of *Chondrus crispus*: the effect of extraction of carrageenan. *Can. J. Bot. 55*: 2053–2064.

McHugh, D.J. 2001. *Prospects for seaweed production in developing countries*. FAO Fisheries Circular 986. Rome, Italy. pp 28.

Muñoz, J., Y. Freile-Pelegrín and D. Robledo. 2004. Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatán, Mexico. *Aquaculture 239*: 161–177.

Pereira, L. and J.F. Mesquita. 2003. Carrageenophytes of occidental Portuguese coast: 1-spectroscopic analysis in eight carrageenophytes from Buarcos bay. *Biomol. Eng. 20*: 223–228.

Pérez-Enríquez, R. 1996. Growth of *Eucheuma isiforme* (C. Agardh) J. Agardh on experimental rafts off the coast of Yucatán state, Mexico. *J. Appl. Phycol. 8*: 27–28.

Piculell, L. 1995. Gelling carrageenans. *In*: (A.M. Stephen, ed.) *Food polysaccharides and their applications*. Marcel Dekker Inc., New York. pp. 205–244.

Robledo, D. 2005. The seaweed resources of México. *In*: (A.T. Critchley and M. Ohno, eds) *Seaweed resources of the world*. JICA, Japan. pp. 331–342.

Rochas, C., M. Lahaye and W. Yaphe. 1986. Sulfate content of carrageenan and agar determined by infrared spectroscopy. *Bot. Mar. 29*: 335–340.

Saito, R. and E.C. de Oliveira. 1990. Chemical screening of Brazilian marine algae producing carrageenans. *Hydrobiologia 204/205*: 585–588.

Santos, G.A. 1989. Carrageenans of species of *Eucheuma* J. Agardh and *Kappaphycus* Doty (Solieriaceae, Rhodophyta). *Aquat. Bot. 36*: 55–67.

Stancioff, D.J. and N.F. Stanley. 1969. Infrared and chemical studies on algal polysaccharides. *Proc. Int. Seaweed Symp. 6*: 595–609.

Stanley, N. 1987. Production, properties and uses of carragee-

nan. *In*: (D.J. McHugh, ed.) *Production and utilization of products from commercial seaweeds*. FAO Fisheries Technical Paper 288. pp. 116–146.

Yakovleva, I.M., I.M. Yermak, E.A. Titlyanov, A.O. Baranova and V.P. Glazunov. 2001. Changes in growth rate, anatomy and polysaccharide content of a sterile form of *Tichocarpus crinitus* (Gmel.) Rupr. (Rhodophyta, Tichocarpaceace) grown under different photon irradiances in the sea of Japan, Russia. *Bot. Mar. 44*: 493–500.

Received 20 June, 2005; accepted 30 November, 2005