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Carrageenan of *Eucheuma isiforme* (Solieriaceae, Rhodophyta) from Nicaragua

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Abstract The yield and physicochemical properties of native and alkali treated carrageenan from *Eucheuma isiforme* harvested from the Nicaraguan coast were investigated. The native carrageenan yield was 57.2% of dry weight and decreased to 43.5% when the alga was alkali treated. Native carrageenan viscosities showed significant differences between native (144.6±3.3 cPs) and treated carrageenan (113.9±2.6 cPs) ($p<0.01$). Alkali treatment reduced carrageenan sulphate content by 19.3% and increased 3,6 AG content by 13%. Alkali-treated carrageenan formed very weak gels in 1.5% solutions (<50 g cm^{-2}). Chemical analysis and FTIR spectra revealed that *Eucheuma isiforme* from Nicaragua is a good source of relatively pure iota-carrageenan with sufficient quality to serve as a substitute for traditional iota-carrageenan sources.

Keywords Carrageenan · *Eucheuma isiforme* · Extraction · Gel properties · Structure

Introduction

During the last 15 years, the carrageenan industry has been growing around 8% per annum producing 28 000 metric tonnes of carrageenan with a value of US$ 270 million (McHugh 2001). Increasing worldwide demand and development of new applications for carrageenan have added urgency to the search for new or additional raw material sources.

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The tropical seaweed *Eucheuma isiforme* (C. Agardh) J. Agardh has been described for the Gulf of Mexico and Caribbean coast (Cheney 1988). Previous studies on reproductive, biochemical aspects and carrageenan have been well documented for populations of *E. isiforme* from Florida (Dawes et al. 1974a, b; Dawes 1977) and recently from the Yucatan coast (Freile-Pelegrin et al. 2006; Freile-Pelegrin and Robledo 2006). The carrageenan content and properties, as well as the biochemical composition, of *E. isiforme* from Yucatan (Gulf of Mexico) differed from those of *E. isiforme* from Florida (US Gulf coast) mainly because of the higher seawater nutrient content on the Yucatan coast (Freile-Pelegrin and Robledo 2006). Environmental factors are known to influence phycocolloid yield and quality (Chopin et al. 1990, Brown 1995), and different physiological and environmental tolerances may also influence variation in carrageenan content. This has been reported for different algae species, between different life stages of the same species (Piriz and Cerezo 1991), and even between individuals of the same species growing under different environmental conditions.

In the Caribbean, *E. isiforme* has been reported as the most important carrageenophyte and was harvested commercially in Belize, Antigua and Barbuda for traditional food applications ('seamoss'). However, this was discontinued as a result of over-exploitation in the 1980s (Smith 1998). In spite of the richness of the Caribbean seaweed flora and the region's proximity to industrial processing facilities in North America and Europe, far less attention has been paid to this species as raw material for the phycocolloid industries, and its use at present has been limited to the preparation of traditional drinks and puddings (Espinosa-Avalos 1994). The scarcity of available data for *E. isiforme* from the Caribbean prevents any assessment of its potential use for carrageenan extraction. In particular, no previous reports on economic important seaweeds in Nicaragua have been produced. During a preliminary evaluation of seaweeds from the Caribbean coast of Nicaragua, *E. isiforme* was the most abundant species identified for potential use.

The present study was intended to improve understanding of carrageenan extracted from *E. isiforme* in preparation for its potential exploitation in Nicaragua. Determining biochemical composition and phycocolloid characteristics may help in understanding the physiological status of the algae, thus providing data for its use as raw material for industry. The carrageenan properties (i.e., yield, gel strength and viscosity) of native and alkali-treated carrageenans are described. Structural analyses were also done, including sulphate, 3,6-anhydro-D-galactose content and infrared spectroscopy.

Materials and methods

Twelve species of macroalgae, including *E. isiforme*, were collected from natural beds at Blue Fields (Nicaragua) in October 2004. The identification of algal material was done according to Wynne (2005). *Eucheuma isiforme* was washed thoroughly with tap water to remove excess salts and sand, oven-dried at 60°C and then milled prior to carrageenan extraction. *Eucheuma isiforme* biomass was free of epiphytes and thus considered 'pure seaweed'.

Ash content was determined according to Dawes (1977). Total protein was determined as proposed by Lowry et al. (1951), and total carbohydrate by the phenol sulphuric acid method (Dubois et al. 1956). All values are presented as percent dry weight.

The carrageenan from *E. isiforme* was obtained using the hot alkaline extraction method described by Freile-Pelegrin et al. (2006). Dry samples (5 g) were rehydrated at room temperature for 12 h in 500 mL of KOH solution (1% w/v), followed by the hot alkali extraction at 85°C during 3 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 slow addition of 250 mL of 2% CTAB (hexadecyl-trimethylamonium bromide) in 9:1 distilled water:acetone (Craigie and Leigh 1978; Chopin et al. 1990) and recovered over paper filter in vacuo. The fibrous carrageenan was carefully washed three times with 63 mL 95% ethanol nearly saturated with sodium acetate to remove CTAB residues. Sodium acetate was removed with three final washings with 95% ethanol and the carrageenan recovered in the same paper filter. The coagulum was dried for 24 h at 60°C, then weighed to calculate percent yield from dry and powdered seaweed. The same procedure without KOH was performed to obtain native carrageenan. All extractions were done in triplicate.

Rheological and chemical analysis

Rheological properties were measured for the native and alkali treated carrageenans. 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 (1 cm^2 plunger). Viscosity was measured using a Cole Parmer Viscosimeter (Vernon Hills, Ill., USA) with a low centipoise adapter at 20 rpm (spindle number 8) on 18-mL samples of a 1.5% carrageenan solution, which 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 1 N HCl at 105°C (Jackson and McCandless 1978). The 3,6 anhydrogalactose content (3,6 AG) was determined following Matsuhiro and Zanlungo (1983). Molar ratios of galactose to 3,6 AG to ester sulfate were calculated based on the total carbohydrate content in the algae. Galactose content is expressed as total carbohydrate content in the algae minus the corresponding 3,6 AG.

Carrageenans extracted were analyzed by Fourier Transformed Infrared spectroscopy (FTIR). A commercial grade, Type II, predominantly iota carrageenan (SIGMA) was used as standard. 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 Thermo-Nicolet Nexus 670 FT-IR spectrometer 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) and were treated statistically by one-way analysis of variance (ANOVA). Carrageenan characteristics from *E. isiforme* collected in Nicaragua and Yucatan were compared using a two-way ANOVA.

Results

The ash, protein and carbohydrate contents for *Eucheuma isiforme* from Nicaragua were 34.8±0.5%, 1.9±0.1% and 58.4±0.5%, respectively. The content and properties of native and alkali-treated carrageenan from *E. isiforme* from Nicaragua are summarized in Table 1. The native carrageenan yield was 57.2% of dry weight. A reduction in yield by 23.9% was observed after alkali treatment. Native and alkali-treated carrageenan formed very weak gels, <50 g cm^{-2} in 1.5% solutions. Carrageenan viscosity decreased after alkali treatment. Alkali treatment reduced carrageenan sulphate content by 19.3% and increased the 3,6 AG content by 13%. This is reflected in the molar ratios obtained for both carrageenans (Table 1). In order to compare carrageenan properties between Nicaraguan and Mexican material, data from Freile-Pelegrin et al. (2006) were also included in Table 1. In this regard, statistical analysis showed that all carrageenan properties had significant differences both between locations and between extraction conditions ($p<0.01$).

FTIR spectra are shown in Fig. 1. All spectra displayed an absorption band at 1,220-1,240 cm^{-1} related to sulphation level (Stancioff and Stanley 1969). The intense signal around 930 cm^{-1} was consistent with the presence of 3,6 AG (Stancioff and Stanley 1969). An increase at this peak was evident between the native and alkali-treated carrageenans implying the presence of the precursor, 1,4-linked galactose-6-sulfate. The spectrum of native carrageenan exhibited a shoulder at 867 cm^{-1}, indicating a sulphate group at C-6, and suggesting the presence of nu-carrageenan, considered the biological precursor to iota-carrageenan (Bodeau-Bellion 1983).

A particularly intense signal was recorded in all samples at 845–847 cm^{-1}, which is assigned to galactose-4-sulphate, and which corroborates the existence of a kappa- and iota-carrageenan mixture (Chopin et al. 1990). Another signal at 803–805 cm^{-1} was attributed to 3,6-anhydrogalactose-2-sulphate and specific to iota carrageenan. The peaks at 805 cm^{-1} and 845 cm^{-1} exhibited slight changes after alkaline transformation. The ratio between 805 and 845 cm^{-1} absorption bands in FTIR spectra was calculated and used as a qualitative parameter to determine the degree of iota/kappa hybridization (Rochas et al. 1986). The treated

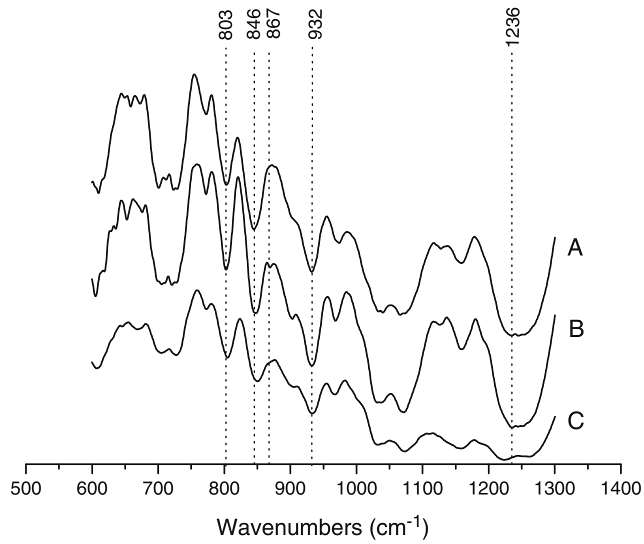

Fig. 1 FTIR spectra of native (**A**) and alkali treated carrageenan (**B**) from *Eucheuma isiforme* from Nicaragua, and commercial iota carrageenan (**C**)

carrageenan and iota standard presented a similar ratio (0.86 and 0.82, respectively) greater than that obtained for native carrageenan (0.63). The increase in the ratio 805/845 in the alkali-treated carageenan corresponded to an increment of the iota faction relatively to kappa fraction.

Discussion

The carrageenan yields showed a comprehensible slight decrease since the hot alkaline extraction operations inevitably involve some degradation of the polysaccharide due to the rigors (heat, alkalinity) of processing (Stanley 1987). In this regard, yield reduction after alkali treatment for *E. isiforme* from Yucatan and Nicaragua were similar (ca. 24%), although the Nicaraguan material showed higher yields when compared to those reported by Freile-Pelegrin et al. (2006) (Table 1).

Table 1 Native and alkali-treated carrageenan properties of *Eucheuma isiforme* from Nicaragua compared with Yucatan

	E. isiforme from Nicaragua		*E. isiforme* from Yucatan	
	Native	Alkali-treated	Native	Alkali-treated
Carrageenan yield (%)	57.2 ± 0.3^{a}	43.5 ± 0.7^{b}	44.5 ± 0.4^{a}	33.8 ± 0.5^{b}
Sulfate (%)	32.6 ± 0.13^{a}	26.3 ± 0.41^{b}	31.0 ± 1.65^{a}	19.6 ± 0.39^{b}
3,6 AG (%)	$19.4\pm0.13^{a*}$	$22.0\pm0.04^{b*}$	27.5 ± 0.30^{a}	34.0 ± 0.89^{b}
Gal:3,AG:Sulfate	1:0.37:1.57	1:0.44:1.36	1:0.73:2.27	1:1.00:1.67
Viscosity (cps)	144.6 ± 3.4^{a}	113.9 ± 2.6^{b}	57.0 ± 0.9^{a}	160.0 ± 0.0^{b}

Carrageenan data from Yucatan (Freile-Pelegrin et al. 2006) included for comparison. Means ± SD indicated
Letters indicate statistical significance between native and alkali treated samples for each location. Different letters indicate significant differences at $p<0.01$; $*p<0.05$

Carrageenan viscosity values were within the ranges reported for other iota-producing species (Azanza-Corrales and Sa-a 1990; Brenden and Bird 1994; Freile-Pelegrin et al. 2006). The present FTIR spectra and chemical analysis results show that alkali treatment converted the precursors into 3,6 AG, diminishing viscosity. This is in agreement with Dawes (1977), who reported higher viscosity values for native carrageenan in *E. isiforme* and *E. nudum*. These authors argued that the high sulphate content in native carrageenan increased hydrophobicity and, therefore, viscosity. Native carrageenan sulphate contents are within the range for iota-producing *Eucheuma* species (Cheney et al. 1987; Santos 1989; Fostier et al. 1992). It is noticeable that reduction in sulphate after alkali treatment was higher for *E. isiforme* from Yucatan than for Nicaraguan specie (Table 1). The 3,6 AG contents in alkali-treated carrageenan from *E. isiforme* from Nicaragua was similar to that reported for Florida *E. isiforme* (19.4%), but lower than those previously reported for the same species (26%) by Fostier et al. (1992) and Freile-Pelegrin et al. (2006). It is also evident that the increase in 3,6 AG contents after alkali conversion was lower for the Nicaraguan *E. isiforme* (Table 1). It is well known that oceanographic and environmental conditions differ substantially between the Yucatan Peninsula and the Caribbean coast, with consequent differences in the physiological responses of seaweeds. A topographic upwelling on the Yucatan shelf has been described as one of the most important upwelling regions on the western oceanic margin (Merino 1997). These conditions may influence physiology and biochemical composition of *E. isiforme* found in Yucatan in a different way to those described for the Caribbean populations.

Very weak carrageenan gels were produced in *E. isiforme* from Nicaragua after alkali treatment (<50 g cm^{-2}). Similar values were reported by Santos (1989) for the same species (53 g cm^{-2}) and by Freile-Pelegrin et al. (2006). The FTIR spectra and molar ratios indicated that the phycocolloids extracted from *E. isiforme* from Nicaragua have a dominant iota-carrageenan which is similar to the same species from Yucatan (Freile-Pelegrín et al. 2006). The Galactose:3,6 AG ratio increased after alkali modification. This pattern has been described by Lawson et al. (1973), 1:1; Dawes (1977) 1:0.4 for the same species. The present FTIR spectra of *E. isiforme* native carrageenan exhibited a shoulder at 867 cm^{-1}, indicating the presence of the precursor nu-carrageenan (Chopin et al. 1990). This was corroborated through chemical analysis that showed an increase in 3, 6 AG content and a reduction in sulphate after alkali modification.

In conclusion, *E. isiforme* from Nicaragua is a good source of relatively pure iota-carrageenan with sufficient quality to serve as a substitute for traditional iota-carrageenan sources.

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