

Chemical screening of Brazilian marine algae producing carrageenans

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Key words: Brazilian algae, carrageenans, phycocolloids, seaweeds

Abstract

The yield, IR spectra, 3,6-anhydro-D-galactose and sulfate content are given for several species of putative carrageenophytes from different sites on the Brazilian coast. Kappa carrageenan was found in *Hypnea musciformis* and *Gigartina* spp.; all the other species tested yield an iota type. The lowest carrageenan yield was found in *Cryptonemia crenulata* (5%), and the highest in *Gigartina* sp. (72%); 3,6-anhydrogalactose content was highest in *Hypnea musciformis* and *Gigartina* spp., whereas sulfate was lowest in *H. musciformis*. Slight variations in the yield of carrageenans were found during the period of observation (March 1988 to May 1989), but no clear pattern could be recognized. No significant variation was observed in plants from different collection sites.

Introduction

Carrageenans are commercially important sulfated galactans extractable with water from certain Rhodophyta. These heavily sulfated polysaccharides (20–50% as OSO₃Na) are large molecules (10⁵–10⁶ daltons). They are composed of alternating 1,3- and 1,4- linked galactose units, which differ in the degree and sites of sulfate esterification (Craigie & Leigh, 1978).

Brazil imports approximately 200 tons of carrageenans per year. The commercial production of carrageenans in Brazil is sporadic and very small. The only carrageenophyte exploited in Brazil, so far, is *Hypnea musciformis* (Wulfen) Lamouroux, harvested on the northeastern coast. In the search for alternative raw materials, species in the families Gigartinaceae, Hypneaceae, Phylloporaceae and Solieriaceae, known elsewhere

as potential sources of carrageenans, were analyzed.

We report the seasonal and geographic variations in carrageenan content of several species collected along the Brazilian coast. The data presented include the yield, infrared spectra, and 3,6-anhydrogalactose and sulfate content.

Materials and methods

Putative carrageenophytes of the families Gigartinaceae, Hypneaceae, Phylloporaceae, and Solieriaceae were collected at four different sites along the Brazilian coast from March 1988 to May 1989. A member of the Cryptonemiaceae, *Cryptonemia crenulata* (J. Agardh) J. Agardh, also was studied.

The plants were collected by hand at low tide

and washed with sea water to remove debris and contaminants. Whenever possible the harvested material was sorted into gametophytic and sporophytic phases.

The samples were frozen with liquid nitrogen and milled in a ball mill. The frozen powder was

placed in a beaker with acetone, heated and stirred for 30 min. The green supernatant was removed by vacuum filtration and discarded. This step was repeated several times. The algal powder was then reextracted with boiling ethanol. The sample was dried in a vacuum oven at 60 °C for

Table 1. Collecting data and carrageenan yields of carrageenophytes. The results are means of three replicates. Data are in % of depigmented dry weight.

Species	Code	Collecting site	Collecting period						
			Mar 1988	Apr-Jun 1988	Jul-Aug 1988	Sep-Nov 1988	Dec 1988 Mar 1989	Apr-May 1989	Av.
<i>Cryptonemia crenulata</i> (J. Agardh) J. Agardh	CRY	J. Pessoa, PB	-	-	-	4.8	-	-	4.8
<i>Eucheuma</i> sp.1	EUC/1	J. Pessoa, PB	-	54.6	-	64.5	-	-	59.6
<i>Eucheuma</i> sp.2	EUC/2	Itamaraca, PE	-	56.8	-	-	58.0	-	57.4
<i>Gigartina teedii</i> (Roth) Lamouroux cystocarpic	GIG/1	Itanhaem, SP	70.4	69.2	69.0	69.2	76.0	70.0	70.7
<i>Gigartina</i> sp.	GIG/2	Recife, PE	-	-	-	71.7	73.0	-	72.4
<i>Gymnogongrus griffithsiae</i> (Turner) Martius	GYM	Ubatuba, SP	46.9	44.3	52.8	56.4	54.8	42.2	49.6
<i>Hypnea musciformis</i> (Wulfen) Lamouroux	HYP/1	Itamaraca, PE	-	56.1	-	59.8	45.8	-	53.9
	HYP/2	J. Pessoa, PB	-	46.7	41.4	47.8	50.4	-	46.6
	HYP/3	Recife, PE	-	-	-	50.0	-	-	50.0
	HYP/4	St. Cruz, ES	-	-	42.3	42.6	54.1	46.4	46.4
	HYP/5	St. Cruz, (cult.)	41.4	27.2	40.4	-	-	-	36.3
	HYP/6	Ubatuba, SP	44.4	39.4	48.6	45.6	52.6	50.1	46.7
<i>Meristiella</i> sp.	MTL/1	Piuma, ES	54.8	-	-	-	-	-	54.8
	MTL/2	Piuma, ES	50.1	-	-	-	-	-	50.1
	MTL/3	J. Pessoa, PB	-	44.0	-	-	-	-	44.0
	MTL/4	J. Pessoa, PB	-	51.0	58.5	-	57.6	-	55.7
<i>Meristiella echinocarpa</i> A (Areschoug) Cheney et Gabrielson cystocarpic	MEC/A1	Itapemirim, ES	-	61.6	-	-	-	-	61.6
tetrasporic	MEC/A2	Itapemirim, ES	-	59.1	-	-	-	-	59.1
<i>Meristiella echinocarpa</i> B (Areschoug) Cheney et Gabrielson cystocarpic	MEC/B1	Itapemirim, ES	-	63.0	-	-	-	-	63.0
tetrasporic	MEC/B2	Itapemirim, ES	-	60.8	-	-	-	-	60.8
vegetative	MEC/B3	Itapemirim, ES	-	56.8	-	-	-	-	56.8
<i>Meristotheca gigartinoides</i> Joly et Ugadim cystocarpic	MTC/1	Itaoca, ES	50.8	61.9	-	-	-	61.2	58.0
tetrasporic	MTC/2	Itaoca, ES	59.2	59.4	-	-	-	59.2	59.3
<i>Solieria filiformis</i> (Kuetzing) Gabrielson	SOL/2	St. Cruz, ES	47.5	45.0	45.0	58.4	56.1	53.0	50.8
	SOL/1	J. Pessoa, PB	-	51.2	-	45.2	47.2	-	47.9

15 h. The extraction and characterization of the carrageenans followed Craigie & Leigh (1978).

Carrageenan yield was determined as percent of algal dry weight. Carrageenans were characterized on the basis of infrared spectra, and 3,6-anhydro-D-galactose content was measured according to the resorcinol reaction as described by Yaphe & Arsenault (1965). Sulfate content was measured by the turbidimetric method of Tabatabai (1974) as modified by Craigie & Wen (1984).

Infrared spectra were obtained by dissolving the material in a few drops of warm distilled water and drying in polyethylene molds. The spectra were recorded on a Shimadzu IR 435 spectrophotometer with the standard conditions for slit program and time drive.

Results and discussion

The data in Table 1 show that the lowest carrageenan yield was found in *Cryptonemia crenulata* (5%), and the highest in *Gigartina* sp. (72%), whereas the other species presented values of 44–70% of the dry weight. *Hypnea musciformis* from a mariculture pilot plant showed a lower carrageenan yield (36%) than other samples of the same species from natural beds.

It is well documented that the production of carrageenans by *Chondrus crispus* Stackhouse is strongly influenced by the season (Black *et al.*, 1965; Fuller & Mathieson, 1972). We, however, did not observe a clear pattern of seasonal or geographic variation in the yield of carrageenans.

Table 2 shows the results of the infrared spectra analysis and 3,6-anhydrogalactose and sulfate contents for 25 samples of carrageenophytes. The highest 3,6-anhydrogalactose content was observed in the carrageenans extracted from species of Hypneaceae and Gigartinaceae. The sulfate content of *Hypnea musciformis* was the lowest of all species analyzed. The infrared spectra showed that *Hypnea* produced kappa carrageenan and *Gigartina* spp. contained kappa and iota carrageenan (Fig. 1).

The carrageenans from *Eucheuma* spp., *Gymnogongrus griffithsiae* (Turner) Martius, *Meristiel-*

Table 2. 3,6-AG and NaSO₃ as % of total carrageenan (± 1 sd) and type of carrageenan (based on infrared spectra) of the species studied. See Table 1 for species names and collecting sites. The data are the means of three replicates.

Native carrageenan			
Species code	3,6-AG	NaSO ₃	Probable type of carrageenan (IR)
CRY	14.5 (± 0.0)	25.6 (± 0.0)	IOTA
EUC/1	20.3 (± 0.7)	30.0 (± 3.3)	IOTA
EUC/2	17.7 (± 0.7)	25.5 (± 1.7)	IOTA
GIG/1	24.5 (± 2.6)	29.3 (± 3.4)	KAPPA-IOTA
GIG/2	27.4 (± 1.7)	27.4 (± 1.7)	KAPPA-IOTA
GYM	19.8 (± 2.6)	27.8 (± 2.2)	IOTA
HYP/1	30.2 (± 0.9)	20.1 (± 1.4)	KAPPA
HYP/2	31.4 (± 2.2)	17.6 (± 2.1)	KAPPA
HYP/3	25.2 (± 0.0)	19.5 (± 0.0)	KAPPA
HYP/4	28.6 (± 2.1)	18.3 (± 2.9)	KAPPA
HYP/5	27.2 (± 3.0)	19.4 (± 0.9)	KAPPA
HYP/6	27.8 (± 2.9)	19.8 (± 4.2)	KAPPA
MTL/1	19.1 (± 0.0)	29.9 (± 0.0)	IOTA
MTL/2	19.0 (± 0.0)	26.0 (± 0.0)	IOTA
MTL/3	18.2 (± 0.0)	25.8 (± 0.0)	IOTA
MTL/4	20.2 (± 1.4)	27.3 (± 4.3)	IOTA
MEC/A1	21.8 (± 0.0)	26.6 (± 0.0)	IOTA
MEC/A2	21.9 (± 0.0)	27.0 (± 0.0)	IOTA
MEC/B1	22.8 (± 0.0)	27.4 (± 0.0)	IOTA
MEC/B2	21.8 (± 0.0)	25.6 (± 0.0)	IOTA
MEC/B3	22.0 (± 0.0)	27.6 (± 0.0)	IOTA
MTC/1	21.3 (± 2.0)	31.4 (± 0.5)	IOTA
MTC/2	21.7 (± 1.7)	29.8 (± 1.5)	IOTA
SOL/1	21.3 (± 2.0)	30.1 (± 5.9)	IOTA
SOL/2	21.7 (± 2.2)	25.0 (± 5.3)	IOTA

la spp., *Meristotheca gigartinoidea* Joly et Ugadim and *Solieria filiformis* (Kuetzing) Gabrielson contained a higher sulfate and lower 3,6-anhydrogalactose content than carrageenans from *Hypnea musciformis*. The iota-type of carrageenan was found in the native carrageenans extracted from species of Phylloporaceae and Solieriaceae. Although we observed a low 3,6-anhydrogalactose content in *Cryptonemia crenulata*, the infrared spectra showed strong evidence for an iota-type of carrageenan in this species (Fig. 1).

No seasonal or geographic variations seemed to influence the type of carrageenan produced.

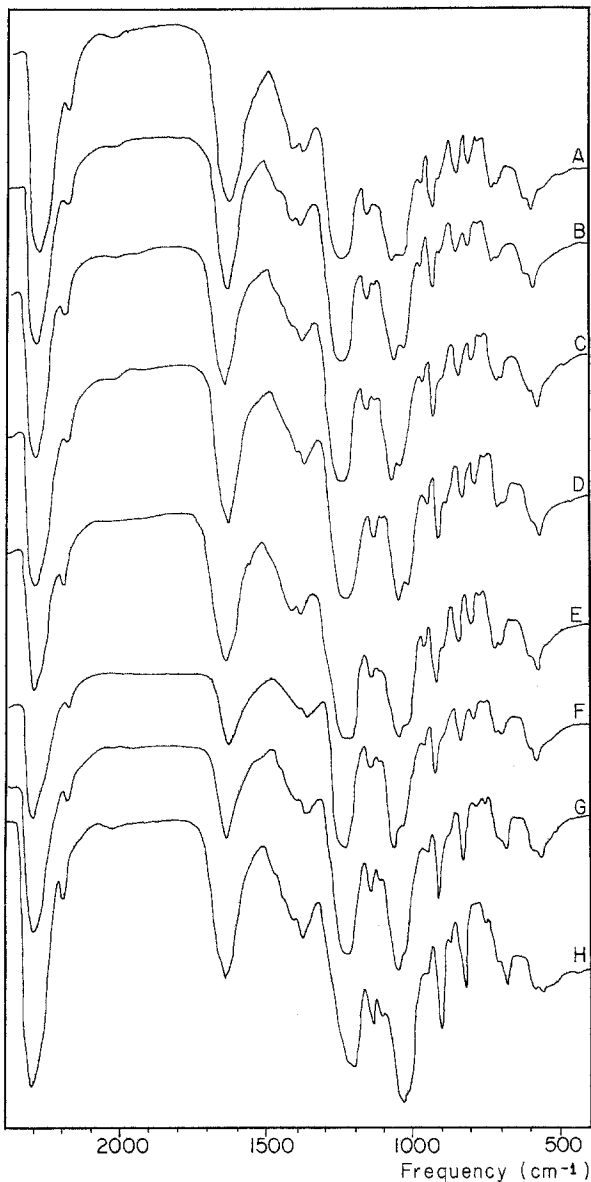


Fig. 1. Infrared spectra of carrageenan from A: *Cryptonemia crenulata*; B: *Eucheuma* sp; C: *Meristiella* sp; D: *Meristotheca gigartinoides*; E: *Solieria filiformis*; F: *Gymnogongrus griffithsiae*; G: *Gigartina* sp; and H: *Hypnea musciformis*.

Acknowledgements

This work was supported by a grant from the Organization of the American States (OAS) and the Financiadora de Estudos e Projetos (FINEP). We thank Dr. J. S. Craigie for his help and Neyla Quege for assistance in collecting the algae.

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