

Carrageenophyte identification by second-derivative Fourier transform infrared spectroscopy

P. J. Cáceres¹, C. A. Faúndez¹, B. Matsuhira^{1*} & J. A. Vasquez²

¹Departamento de Ciencias Químicas, Facultad de Química y Biología, Universidad de Santiago de Chile, casilla 5659, Santiago 2, Chile ²Departamento de Biología Marina, Universidad Católica del Norte, casilla 117, Coquimbo, Chile (* Author for correspondence)

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Abstract

The second-derivative mode of the Fourier transform I.R. spectra of dried algal material has been applied to distinguish the carrageenans-producing *Stenogramme interrupta* from the isomorphous species *Rhodymenia howeana*. Spectra of the tetrasporophyte *S. interrupta* showed bands assigned to a λ -carrageenan type polysaccharide, while the gametophytic and cystocarpic plants showed the characteristic absorptions of κ - and ι -carrageenans. Results were confirmed by hot water extraction of samples of the three nuclear phases of *S. interrupta* and characterization of the extracts by chemical analysis.

Introduction

Stenogramme interrupta (C. Ag.) Mont., 1846 (Phylloporaceae, Gigartinales) and *Rhodymenia howeana* Dawson, 1941 (Rhodymeniaceae, Rhodymeniales) are two Rhodophyta species with few phylogenetic relationships. However, both species are morphologically identical. Taxonomic identification is impossible without the presence of reproductive structures. *S. interrupta* presents an isomorphic trifasic reproductive strategy. The three reproductive phases occur simultaneously in nature. Both species are common in coastal sheltered areas and have a complete overlap in shallow subtidal habitats of Perú and northern Chile (Ramírez & Rojas, 1986; Santelices 1989).

Carrageenans are cell-wall polysaccharides extracted from members of the order Gigartinales; they have a repeating backbone unit of alternating 3-O-linked β -D-galactopyranosyl and 4-O-linked α -D-galactopyranosyl residues. Members of this family differ in the content and position of hemi-ester sulfate and in the content of 3,6-anhydro-D-galactopyranose (Painter, 1983).

Infrared spectroscopy has been applied for many years in the characterization of sulfated polysaccha-

rides from seaweeds (e.g. Anderson et al., 1968; Stancoff & Stanley, 1969; Whyte et al., 1985). With the advent of Fourier transform infrared spectroscopy (FT-IR) new applications were developed. For example, FT-IR in the second derivative mode was applied to differentiate between agar- and carrageenan-types seaweed galactans (Matsuhira & Rivas, 1993) and Chopin & Whalen (1993) identified carrageenans by FT-IR diffuse reflectance spectroscopy directly on dried algal material. The second-derivative mode of FT-IR spectra is useful in distinguishing agar-producing from carrageenan-producing algal material (Matsuhira, in press).

McCandless et al. (1982) analysed by IR spectroscopy and immunochemical reactivity the polysaccharides obtained by extraction with 0.5 M NaHCO₃ of members of the Phylloporaceae. Gametophytes of *Stenogramme interrupta* yielded κ - ι hybrid carrageenan while tetrasporophytes yielded λ -carrageenan. On the other hand, Furneaux and Miller (1985) reported that the aqueous extract from carposporic *S. interrupta* was 75% floridean starch and the remaining polysaccharide after enzymic hydrolysis did not show a carrageenan structure.

Some members of the Rhodophyta produce xylans as the major polysaccharides (Painter, 1983; Percival & McDowell, 1967). In many cases, fractionation of the aqueous extracts also affords sulfated polysaccharides. Usov et al. (1978) isolated a sulfated galactan and a family of β -D-xylans with variable proportions of 1 \rightarrow 3 and 1 \rightarrow 4 linkages from *Rhodymenia stenogona* (*Palmaria stenogona*). By extractions in different conditions, *Galaxaura squalida* yielded a sulfated xylogalactomannan and several neutral xylan fractions (Usov & Shashkov, 1985). Matulewicz et al. (1994) reported that *Nothogenia fastigiata* synthesizes neutral xylans as well as sulfated xylogalactans. Fractionation with cetrimide of the aqueous extract of *Palmaria decipiens* (*Leptosomia simplex*) yields a neutral xylan and a sulfated xylogalactan (Jerez et al., in press; Matsuhira & Urzúa, in press).

In this work, the application of the second-derivative FT-IR spectroscopy to directly identify a carrageenan producing seaweed is presented.

Materials and methods

Samples of *Stenogramme interrupta* and *Rhodymenia howeana* were collected seasonally at La Herradura Bay (29° 71'S, 71° 21'W) using SCUBA. In the laboratory the samples were sorted by species and nuclear phases. Specimens with different phenologies were deposited in Sala de Sistemática y Colecciones of Universidad Católica del Norte, Coquimbo, Chile.

FT-IR spectroscopy

Samples of seaweeds were ground in a Moulinex Moulinette Chopper 320 and dried at 65 °C for 8 h *in vacuo*. The polysaccharides were powdered under liquid nitrogen and also dried for 8 h at 65 °C *in vacuo*. The FT-IR spectra of the algal samples and polysaccharides in KBr pellets (10% w/w) were recorded in the 4000–400 cm^{-1} region using a Bruker IFS 66 v instrument; 32 scans were taken with a resolution of 2 cm^{-1} . Derivation, including Savitzky-Golay algorithm (Maddam & Mead, 1982) with 23 smoothing points was performed using the OPUS/I.R. version 1.44 software incorporated into the hardware of the instrument.

Sulfate content

Sulfate content was determined by microanalysis in Facultad de Química y Farmacia, Universidad de Chile.

Extraction

Samples of *S. interrupta* collected in December were extracted with water at 95 °C (Matsuhira & Urzúa, 1992).

Double hydrolysis-reduction

Samples of polysaccharides were hydrolysed according to Stevenson and Furneaux (1991). The constituent-sugars were analysed by gas-liquid chromatography (GLC) and gas-liquid chromatography-mass spectrometry. GLC was carried out in a Shimadzu GC-14B gas chromatographed equipped with a flame ionization detector using a fused silica gel capillary column (15 m \times 0.25 mm) coated with SP-2330 and performed with an initial 5 min hold at 150 °C and then at 5 °C min^{-1} to 210 °C for 10 min. The helium flow was 20 mL min^{-1} . The identities of the acetyl alditols were determined by GLC-mass spectra in a Hewlett-Packard 5890 gas-liquid chromatograph coupled to a Trio-2 VG Masslab mass spectrometer.

Results and discussion

Representatives samples of normal and second-derivative FT-IR spectra of seaweed samples are shown in Figures 1–3.

The normal spectrum (Figure 1A) of cystocarpic *S. interrupta* shows a peak at 932.1 cm^{-1} assigned to the presence of the 3,6-anhydrogalactopyranosyl ring and a weak signal at 874.4 cm^{-1} . Its second derivative spectrum (Figure 1B) shows in the same region more bands, at 965.6 cm^{-1} assigned to the vibrational motion of the glycosidic linkage, at 931.8 cm^{-1} due to the 3,6-anhydro ring, at 900.8 cm^{-1} assigned to β -D-galactopyranosyl units and the band at 871.5 cm^{-1} attributed to equatorial C-H deformation other than anomeric ones (Mathlouthi & Koenig, 1986). The absorptions at 841.2 and 803.5 cm^{-1} are assigned to axial hemi-ester sulfate on C-4 of galactopyranosyl residues and to sulfate groups on C-2 of 3,6-anhydro-D-galactopyranosyl residues, respectively. The bands at 777.6 and 739.7 cm^{-1} are assigned to the skeletal bending of the pyranose ring and those at 615.2 and 578.3 cm^{-1} to the S-O stretching vibration (Matsuhira, in press). The normal FT-IR spectrum (Figure 2A) of tetrasporic *S. interrupta* showed a shoulder around 930 cm^{-1} and a broad band centered at 843.2 cm^{-1} assigned to 4-sulfate group. This band, in the sec-

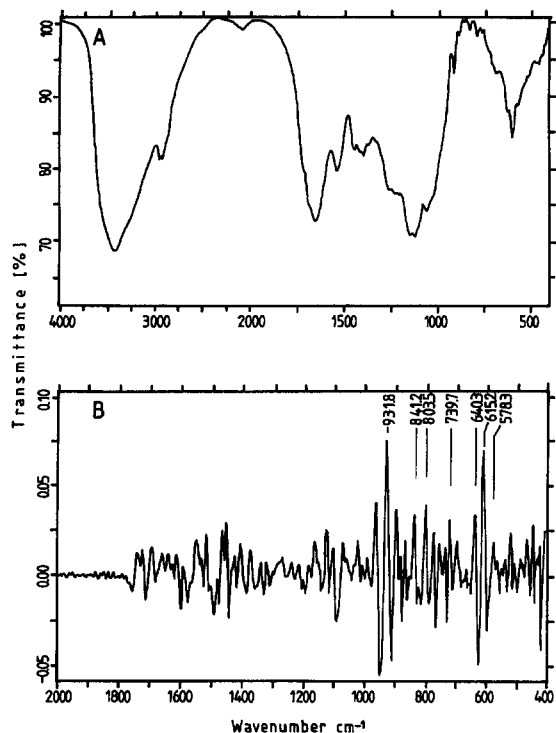


Figure 1. FT-IR spectra of cystocarpic *Stenogramme interrupta*. A: normal. B: second-derivative

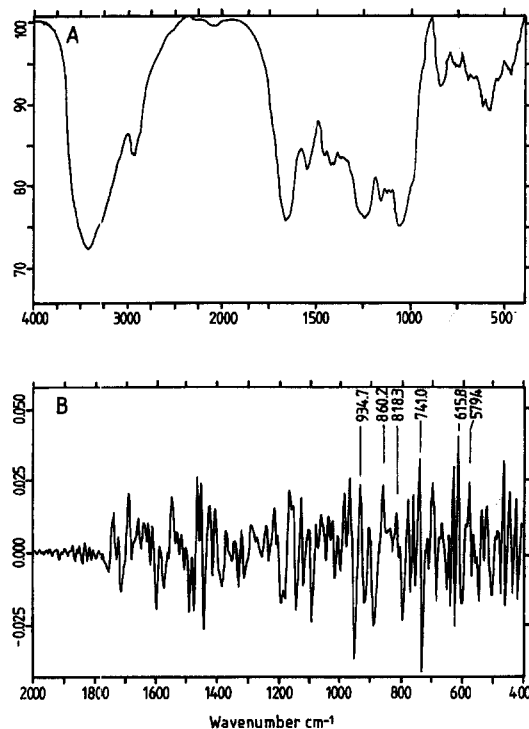


Figure 2. FT-IR spectra of tetrasporic *Stenogramme interrupta*. A: normal. B: second-derivative.

ond derivative mode (Figure 2B) is resolved into two bands at 860.7 cm^{-1} and at 815.8 cm^{-1} . The latter was assigned to equatorial primary sulfate group. The second-derivative spectra of both samples exhibited in the region $1160\text{--}1000\text{ cm}^{-1}$ the characteristic bands of carrageenans (Matsuhiro & Rivas, 1993).

FT-IR spectra of *Rhodymenia howeana* do not exhibit significant absorption bands between $1100\text{--}900\text{ cm}^{-1}$. The FT-IR spectrum in the second derivative mode of the cystocarpic plants (Figure 3B) presents a band at 872 cm^{-1} assigned to the deformation of α anomeric C-H and two bands in the region $860\text{--}840$ which may be assigned to sulfate groups.

Hot water extraction of gametophytic, cystocarpic and tetrasporophytic samples of *S. interrupta* yielded polysaccharides representing 20.7%, 18.9% and 18.1% dry weight, respectively. The sulfate contents for the polysaccharides from gametophytic, cystocarpic and tetrasporic plants were 20.85, 21.40, and 22.60%, respectively. These values are in accord with those for the carrageenans from *S. interrupta* in California (McCandless et al., 1982). Figure 4 shows the FT-IR spectrum of the polysaccharide from cystocarpic plants and the second derivative spectrum. Both spectra are

very similar to those of Figure 1, which correspond to the algae. Table 1 shows the characteristic bands of the second derivative FT-IR spectra of dried, ground samples of *S. interrupta* and *R. howeana* collected in summer, and those of the polysaccharides extracted from *S. interrupta*. No significant variations were found for the absorption values in the spectra (not shown) of the samples of *S. interrupta* and *R. howeana* collected in the other three seasons.

The IR absorptions of the polysaccharide extracted from gametophytic *S. interrupta* are very similar to those of the polysaccharide from cystocarpic plants and also to the values for the algal samples. They show the characteristic absorptions bands (peaks at around 930 , 840 and 805 cm^{-1}) of κ - ι carrageenan structures. The results obtained by FT-IR spectroscopy were confirmed by chemical characterization of the extracts from *S. interrupta*. Double hydrolysis followed by GLC analysis showed that the galactose:3,6-anhydrogalactose molar ratio was 1.00:0.77 for the polysaccharide from gametophytic plants and 1.00:0.50 for the polysaccharide from cystocarpic plants. These results are well with the values reported for κ - ι carrageenan type polysaccharides

Table 1. Second-derivative FT-IR frequencies (cm^{-1}) of seaweeds and polysaccharides in the 1000–500 cm^{-1} region

| | | | | | | | | | | | |
|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1* | 967.0 | 932.1 | 901.2 | 874.7 | 843.5 | 803.9 | 778.0 | 746.4 | 716.4 | 618.3 | 582.0 |
| 2 | 965.6 | 931.8 | 900.8 | 871.1 | 841.2 | 803.5 | 776.6 | 739.7 | 723.8 | 615.2 | 578.3 |
| 3 | 970.3 | 934.7 | 902.0 | 860.7 | 818.3 | | 777.8 | 761.1 | 741.0 | 615.8 | 579.4 |
| 4 | 964.5 | 933.6 | 902.4 | 886.0 | 853.6 | 835.2 | 807.3 | 774.9 | 727.6 | | 584.3 |
| 5 | 963.2 | 933.8 | 902.1 | 886.3 | 852.5 | 836.0 | 806.5 | 774.7 | 726.4 | 688.0 | 574.6 |
| 6 | 971.4 | 934.8 | | | | | 776.6 | 764.0 | 702.4 | 617.6 | 580.4 |
| 7 | | | | 872.9 | 858.8 | 844.6 | 777.9 | 761.2 | 714.1 | 658.4 | 643.7 |
| 8 | | | | 873.3 | 859.7 | 843.2 | 815.8 | 768.5 | 743.5 | 699.2 | 617.1 |

*1 = gametophytic *Stenogramme interrupta*, 2 = cystocarpic *S. interrupta*, 3 = tetrasporic *S. interrupta*, 4 = polysaccharide from gametophytic *S. interrupta*, 5 = polysaccharide from cystocarpic *S. interrupta*, 6 = polysaccharide from tetrasporic *S. interrupta*, 7 = cystocarpic *Rhodymenia howeana*, 8 = tetrasporic *R. howeana*.

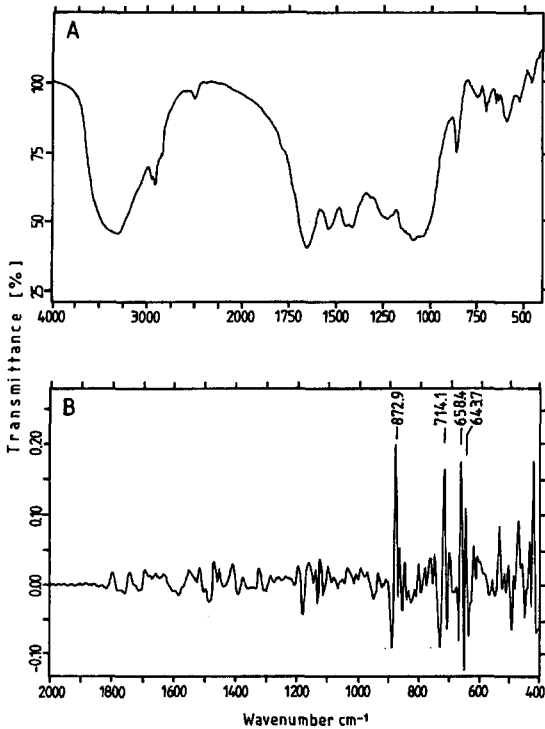


Figure 3. FT-IR spectra of cystocarpic *Rhodymenia howeana*. A: normal. B: second derivative.

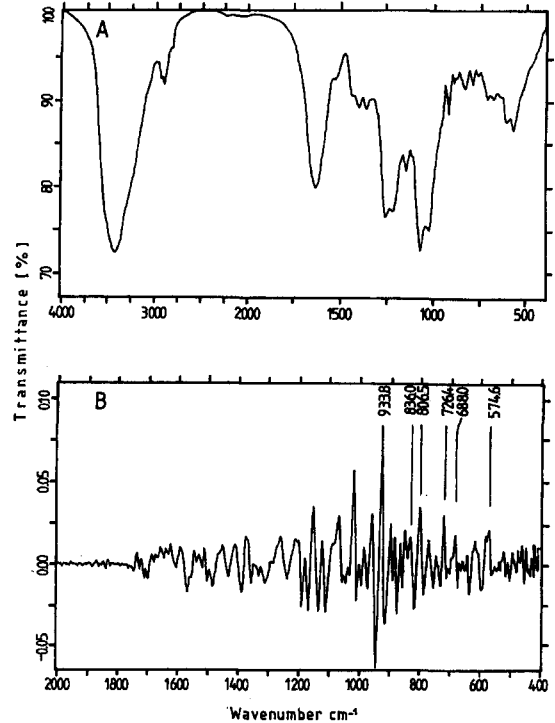


Figure 4. FT-IR spectra of the polysaccharide from cystocarpic *Stenogramme interrupta*. A: normal. B: second-derivative.

(Anderson et al., 1973; Lawson et al., 1973). The molar ratio (1.00:0.16) found for the polysaccharide from tetrasporophytes indicates the presence of a λ -carrageenan type structure.

On the other hand, total hydrolysis of the sequential extraction products from tetrasporic and cystocarpic *R. howeana* indicated that both phenotypes produce complex mixtures of sulfated xylogalactans, floridean

starch and xylans (C. A. Faúndez, B. Matsuhiro & J. A. Vásquez, unpublished data).

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References

- Anderson NS, Dolan TCS, Penman A, Rees DA, Muller GP, Stancioff DJ, Stanley NF (1968) Carrageenans IV. Variation in the structure and gel properties of κ -carrageenan and the characterization of sulfate esters by infrared spectroscopy. *J. chem. Soc. C*: 602–606.
- Anderson NS, Dolan TCS, Rees DA (1973) Carrageenans. Part VII. Polysaccharides from *Eu cheuma spinosum* and *Eu cheuma cottonii*. The covalent structure of *t*-carrageenan. *J. chem. Soc. Perkin I*: 2173–2176.
- Chopin T, Whalen E (1993) A new and rapid method for carrageenan identification by FT IR diffuse reflectance spectroscopy directly on dried, ground algal material. *Carbohydr. Res.* 246: 51–59.
- Fur neaux RH, Miller IJ (1985) Water soluble polysaccharides from the New Zealand red algae in the family Phylloporaceae. *Bot. mar.* 28: 419–425.
- Jerez JR, Matsuhiro B, Urzúa CC (in press) Chemical modifications of the xylan from *Palmaria decipiens*. *Carbohydr. Polym.*
- Lawson CJ, Rees DA, Stancioff DJ, Stanley NF (1973) Carrageenans. Part VIII. Repeating structures of galactan sulphates from *Gigartina fastigiata*, *Gigartina canaliculata*, *Gigartina chamissoi*, *Gigartina atropurpurea*, *Anhfeldtia durvillaei*, *Gymnongrus furcellatus*, *Eu cheuma cottonii*, *Eu cheuma spinosum*, *Eu cheuma isiforme*, *Eu cheuma uncinatum*, *Aghardiella tenera*, *Pachymenia hymantophora*, and *Gloiopeltis cervicornis*. *J. chem. Soc. Perkin I*: 2177–2182.
- Maddam WF, Mead WL (1982) The measurement of derivative i.r. spectra-I. Background studies. *Spectrochim. Acta* 38A: 437–444.
- Mathluothi M, Koenig JL (1986) Vibrational spectra of carbohydrates. *Adv. Carbohydr. Chem.* 44: 7–66.
- Matsuhiro B (in press) Vibrational spectroscopy of seaweed galactans. *Hydrobiologia*
- Matsuhiro B, Rivas P (1993) Second-derivative Fourier transform infrared spectra of seaweed galactans. *J. appl. Phycol.* 5: 45–51.
- Matsuhiro B, Urzúa CC (1992) Heterogeneity of carrageenans from *Chondrus crispus*. *Phytochemistry* 31: 531–534.
- Matsuhiro B, Urzúa CC (in press) The acidic polysaccharide from *Palmaria decipiens*. *Hydrobiologia*.
- Matulewicz MC, Haines HH, Cerezo AS (1994) Sulphated xylogalactans from *Nothogenia fastigiata*. *Phytochemistry* 36: 97–103.
- McCandless EL, West JA, Guiry MD (1982) Carrageenan patterns in the Phylloporaceae. *Biochem. Syst. Biol.* 10: 275–284.
- Painter TJ (1983) Algal polysaccharides. Aspinnall GO (ed.) In: *The Polysaccharides Vol. 2*. Academic Press, New York: 195–285.
- Percival E, McDowell RH (1967) *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, London: 88–91.
- Ramírez ME, Rojas G (1986) El género *Stenogramme* (Rhodophyta, Gigartinales) en la costa temperada del Pacífico sur-oriental. Westermeier R (ed.). In: *Actas del segundo congreso sobre algas marinas chilenas*. Universidad Austral de Chile, Valdivia: 191–200.
- Santelices B (1989) *Algas Marinas de Chile*, Ediciones Universidad Católica de Chile, Santiago: 337–343.
- Stancioff DJ, Stanley NF (1969) Infrared and chemical studies on algal polysaccharides. *Proc. int. Seaweed Symp.* 6: 595–609.
- Stevenson TT, Fur neaux RH (1991) Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydr. Res.* 210: 277–298.
- Usov AI, Yarostsky SV, Estevez ML (1981) Polysaccharides of algae. XXXII. Polysaccharides of the red seaweed *Galaxaura squalida* Kjellm. *Bioorg. Khim.* 7: 1261–1270. (in Russian)
- Usov AI, Yarostsky SV, Shashkov AS, Tishchenko VP (1978) Polysaccharides of algae. XXII. polysaccharide composition of *Rhodomymeria stenogona* Perest and ^{13}C -NMR spectroscopy application for elucidation of xylan structures. *Bioorg. Khim.* 4: 57–65.
- Whyte JNC, Hosford SPC, Englar JR (1985) Assignment of agar or carrageenan structures to red algal polysaccharides. *Carbohydr. Res.* 140: 336–341.