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Carrageenophytes of occidental Portuguese coast: 1-spectroscopic analysis in eight carrageenophytes from Buarcos bay

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Abstract

Infrared and Raman spectroscopic analysis of the carrageenan (alkaline extraction) in eight species (representing seven genera and four families) of Gigartinales, in different reproductive phases from Buarcos bay (Figueira da Foz, Portugal), were studied. Female gametophytes and non-fertile thalli samples of *Chondrus crispus*, *Mastocarpus stellatus*, *Chondracanthus teedei* var. *lusitanicus*, *Gigartina pistillata* and *Chondracanthus acicularis* present a κ-carrageenan profile or varying degrees of a κ–ι hybrid. The presence of κ–ι hybrid carrageenan in *C. teedei* var. *lusitanicus* was confirmed by ^{13}C NMR. The carrageenans extracted from *Gymnogongrus crenulatus* and *Ahnfeltiopsis devoniensis* are constituted mainly by ι-carrageenan but seasonal variations in the nature of carrageenans are present. λ-Family carrageenans were found in tetrasporophytes of *C. crispus*, *M. stellatus*, *C. teedei* var. *lusitanicus*, *C. acicularis* and *G. pistillata*. *Calliblepharis jubata* presents carrageenans of ι-type in all reproductive stages.

Keywords: Portugal; Carrageenophytes; Carrageenan; FTIR; FT-Raman; ^{13}C NMR

1. Introduction

The carrageenophytes pertaining to the Gigartinaceae, Petrocelidaceae, Phyllophoraceae and Cystocloniaceae families (Gigartinales, Rhodophyta) are widely distributed in the Atlantic centre and north coast of Portugal. However, *Mastocarpus stellatus* (Petrocelidaceae) and *Chondrus crispus* (Gigartinaceae) are the only species currently harvested for industrial aims, mainly in the north coast (Viana do Castelo) (personal observation).

Carrageenan is a structural cell wall component constituted by sulphated polysaccharides (galactans), which can form gels in water or milk solutions. This phycocolloid is used mainly in cosmetic, pharmaceutical and food industry [1].

The κ-carrageenan and the hybrid forms (κ–ι) occur normally in Gigartinaceae and Petrocelidaceae gametophytes; the λ-family carrageenans appear habitually in the tetrasporophytic stages [2]. Finally, the ι-carrageenan (and ι-κ hybrids) is produced mainly by species of the genus *Eucheuma* (*Eucheuma denticulatum*) and also by some other species of the Cystocloniaceae and Phyllophoraceae families [2,3].

2. Materials and methods

Samples of all eight studied carrageenophytes were collected randomly in the intertidal zone, at different times (Table 1), in Buarcos Bay (Figueira da Foz, Portugal). The seaweeds were collected by hand at low tide and washed with distillate freshwater to eliminate salt, debris and contaminants. The material was separated, whenever possible, into three groups (female gametophytes, tetrasporophytes and non-fertile thalli) and dried to constant weight at 60 °C.

The procedure for carrageenan extraction (alkaline extraction) has been previously outlined [4].

The carrageenan samples have been analysed by FTIR, FT-Raman (only for the λ-family carrageenans) and ^{13}C NMR (only for the κ–ι hybrid samples) spectroscopy.

The FTIR spectra were recorded on an IFS 55 spectrometer, using a Golden Gate single reflection

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Table 1
Species, reproductive stages, sampling dates and carrageenan type

Code	Species	Stages	Dates	Carrageenan type
A	*C. crispus* Stackhouse	NF	December 2000	κ
B	*C. crispus* Stackhouse	FG	February 2001	κ
C	*M. stellatus* (Stackhouse) Guiry	G	August 2001	κ
D	*G. pistillata* (S.G. Gmelin) Stackhouse	FG	March 2002	κ (ι)
E	*C. teedei* var. *lusitanicus* (Rodrigues) Bárbara and Cremades	NF	June 2001	κ–ι
F	*C. teedei* var. *lusitanicus* (Rodrigues) Bárbara and Cremades	FG	June 2001	κ–ι
G	*A. devoniensis* (Greville) P.C. Silva and DeCew	G	July 2001	ι (κ)
H	*A. devoniensis* (Greville) P.C. Silva and DeCew	NF	August 2001	ι (κ)
I	*G. crenulatus* (Turner) J. Agardh	TB	April 2002	ι (κ)
J	*A. devoniensis* (Greville) P.C. Silva and DeCew	G	December 2001	ι (κ)
L	*C. jubata* (Goodenough and Woodward) Kützing	NF	March 2001	ι
M	*C. jubata* (Goodenough and Woodward) Kützing	T	May 2001	ι
N	*C. jubata* (Goodenough and Woodward) Kützing	FG	April 2001	ι
O	*G. crenulatus* (Turner) J. Agardh	TB	November 2001	ι
Q	*C. crispus* Stackhouse	T	May 2001	λ
R	*G. pistillata* (S.G. Gmelin) Stackhouse	H	February 2002	λ
S	*G. pistillata* (S.G. Gmelin) Stackhouse	T	April 2002	λ
T	*Chondrachantus acicularis* (Roth) Fredericq	T	August 2001	ξ
U	*C. teedei* var. *lusitanicus* (Rodrigues) Bárbara and Cremades	T	June 2001	ξ

T, tetrasporophytes; FG, female gametophytes; G, gametophytes; NF, non-fertile thalli; TB, tetrasporoblastic thalli; H, heterosporic thalli.

diamond ATR system, with no need for sample preparation. All spectra are the average of two counts, with 128 scans each and a resolution of 2 cm^{-1}.

The room temperature FT-Raman spectra were recorded on a RFS-100 Bruker FT-spectrometer using a Nd:YAG laser with excitation wavelength of 1064 nm. Each spectrum is the averaging of two repeated measurements of 150 scans each and 2 cm^{-1} resolution.

^{13}C NMR spectra were recorded on a Varian Unity 500 spectrometer at 125.69 MHz. Samples (15/20 mg ml^{-1}) were dissolved in D_2O and spectra recorded at 80 °C, 10 000 accumulations, pulse 15 μs, acquisition time 3 s and relaxation delay 5 s. The chemical shifts (ppm) were measured in relation to the reference acid sodium salt (TMSPSA).

3. Results

Information about sampling dates, reproductive stages and carrageenan type of the analysed algal species is given in Table 1.

The FTIR spectra of the *C. crispus*, *M. stellatus*, *Gigartina pistillata* (Gigartinaceaea) gametophytes samples and non-fertile thalli samples of *C. crispus* (Fig. 1) present strong absorption bands in 930 cm^{-1} region (3,6-anhydro-D-galactose) and in the 845 cm^{-1} region (D-galactose-4-sulphate), typical of κ-carrageenan. They present lower absorbance in the 805 cm^{-1} region (3,6-anhydro-D-galactose-2-sulphate), which indicates a presence of low quantities of ι-carrageenan [5,6]. The ratio between 805 and 845 cm^{-1} absorption bands was calculated [5,7] and used as parameter to determine the degree of the κ–ι hybridisation (Figs. 1, 3 and 4).

In the *Chondracanthus teedei* var. *lusitanicus* (Gigartinaceae) gametophytes and non-fertile thalli samples, the FTIR spectra (Fig. 2) present strong absorption in 930 and 845 cm^{-1} and medium absorption in 805 cm^{-1} bands. The presence of three picks in the anomeric zone, in ^{13}C NMR spectra (Table 2), is typical of κ–ι hybrid carrageenan [8].

For the species *Ahnfeltiopsis devoniensis* (Phyllophoraceae), *Gymnogongrus crenulatus* (Phyllophoraceae) and *Calliblepharis jubata* (Solieriaceae), FTIR spectra show absorption bands at 930, 845 and 805 cm^{-1}, which represent the characteristic triplet for ι-carrageenan (Figs. 3 and 4). However, the peak ratio (805/845 cm^{-1}) is lower in *A. devoniensis* and *G. crenulatus* than the presented by the *C. jubata* and *E. denticulatum* (sample from Sigma, C-4014).

In the *C. crispus* and *G. pistillata* tetrasporic samples the FTIR spectra (Fig. 5) present broad absorption bands in 820–830 cm^{-1} region, characteristic of the λ-carrageenan [5,6]. The FT-Raman spectra of these samples present a broad peak in 820 cm^{-1} (Fig. 6), which confirm the presence of λ-carrageenan.

The FTIR spectra of tetrasporic samples of *Chondracanthus* species show sharper peaks at 830 cm^{-1}, but little absorption at 820 cm^{-1}, which indicates the presence of ξ-carrageenan (Fig. 5). The presence of 815 and 850 cm^{-1} in FT-Raman spectra confirms the presence of the mentioned carrageenan (Fig. 7).

4. Discussion

In this study the κ-family carrageenans (κ and κ–ι hybrids) are produced by the gametophytes and the non-fertile thalli of the species belonging to the Gigartinaceae and Petrocelidaceae families, while the λ-family carrageenans (λ and ξ carrageenans) are produced by the tetrasporic stages (Table 1).

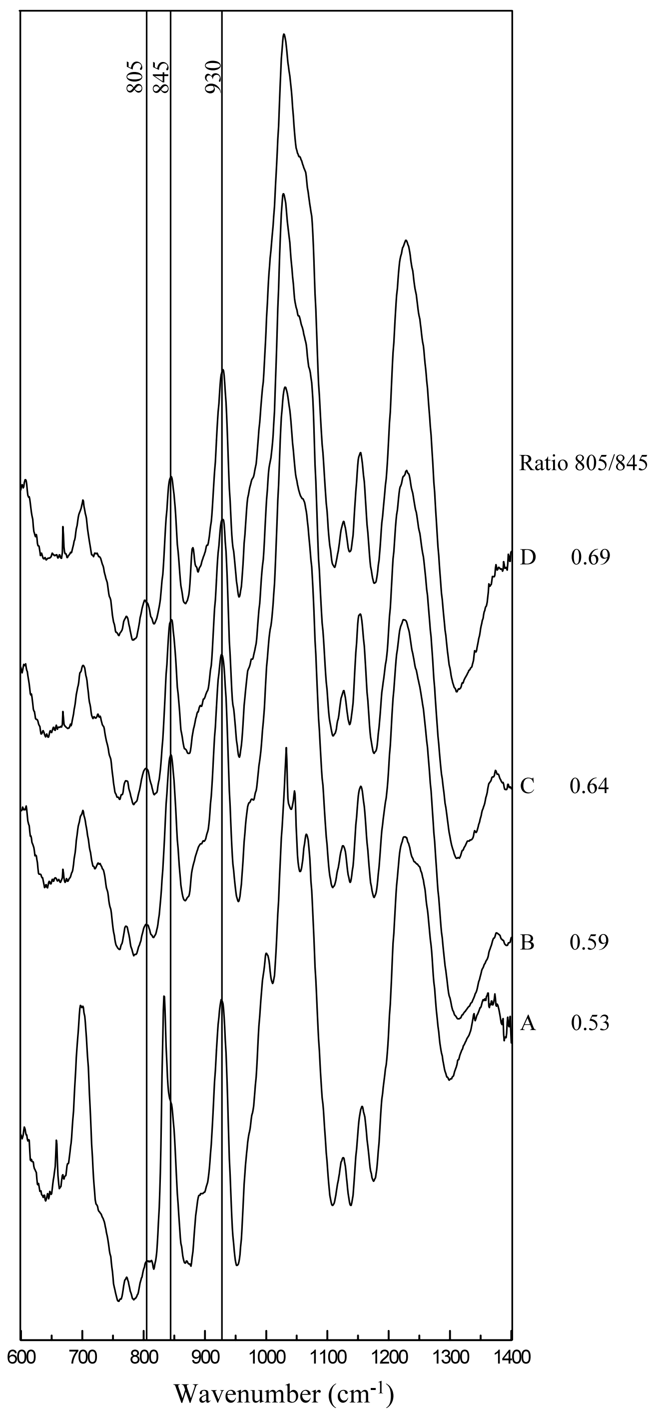

Fig. 1. FTIR spectra of alkali treated carrageenan: (A) *C. crispus* (non-fertile thalli), (B) *C. crispus* (female gametophytes), (C) *M. stellatus* (gametophytes), (D) *G. pistillata* (female gametophytes).

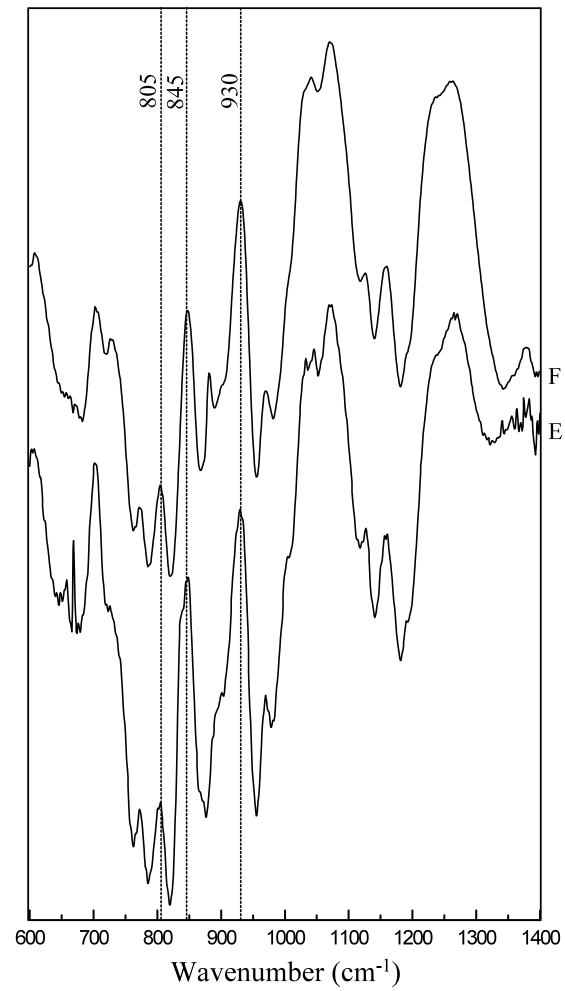

Fig. 2. FTIR spectra of alkali treated carrageenan: (E) *C. teedei* var. *lusitanicus* (non-fertile thalli), (F) *C. teedei* var. *lusitanicus* (female gametophytes).

Table 2
^{13}C NMR chemical shifts for *C. teedei* var. *lusitanicus* carrageenans: E (non-fertile thalli) and F (female gametophytes)

E	F	Assignment
104.7	104.7	ιG_1; κG_1
97.6	97.5	κA_1
94.3	94.3	ιA_1
81.4	81.2	κA_3; κG_3
80.6	80.6	ιA_4; κA_4
79.0	79.1	ιG_3; κA_5
77.0	77.0	ιG_5; κG_5; ιA_2
76.4	76.3	κG_4
74.3	74.4	ιG_4
72.1	71.9	ιA_6; κG_2; κA_2
63.5	63.5	ιG_6; κG_6

G, galactose; A, anhydrogalactose.

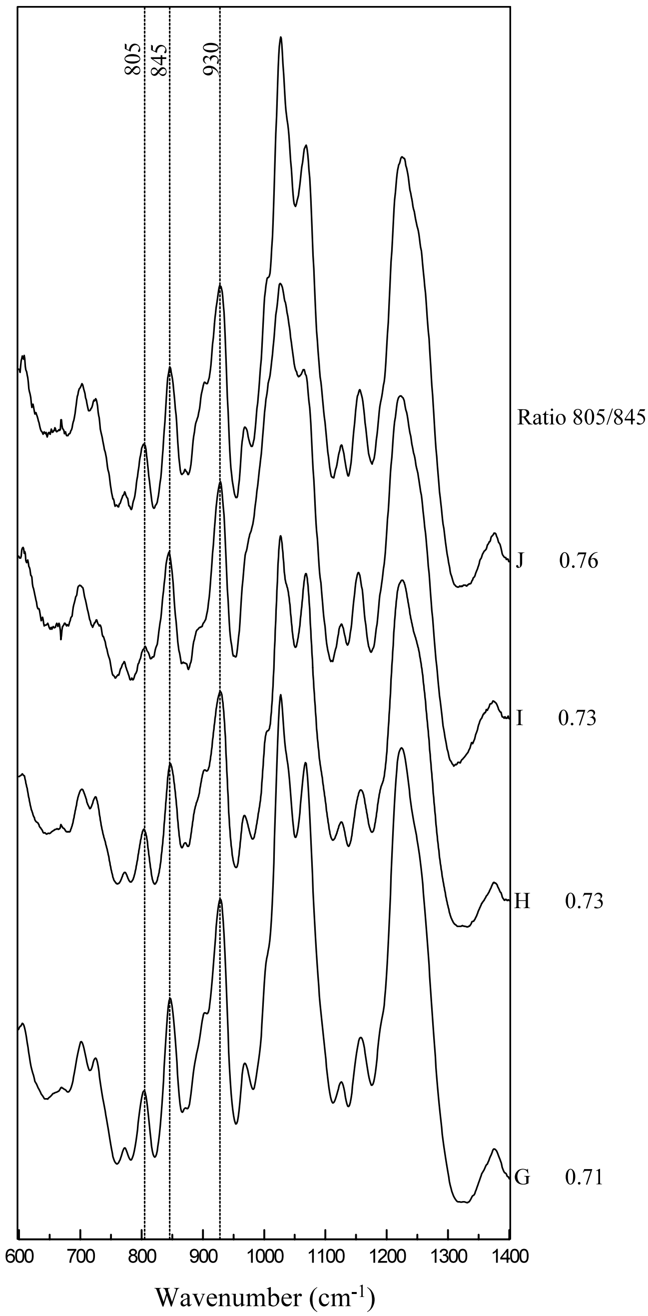

Fig. 3. FTIR spectra of alkali treated carrageenan: (G) *A. devoniensis* (gametophytes), (H) *A. devoniensis* (non-fertile thalli), (I) *G. crenulatus* (tetrasporoblastic thalli) (J) *A. devoniensis* (gametophytes).

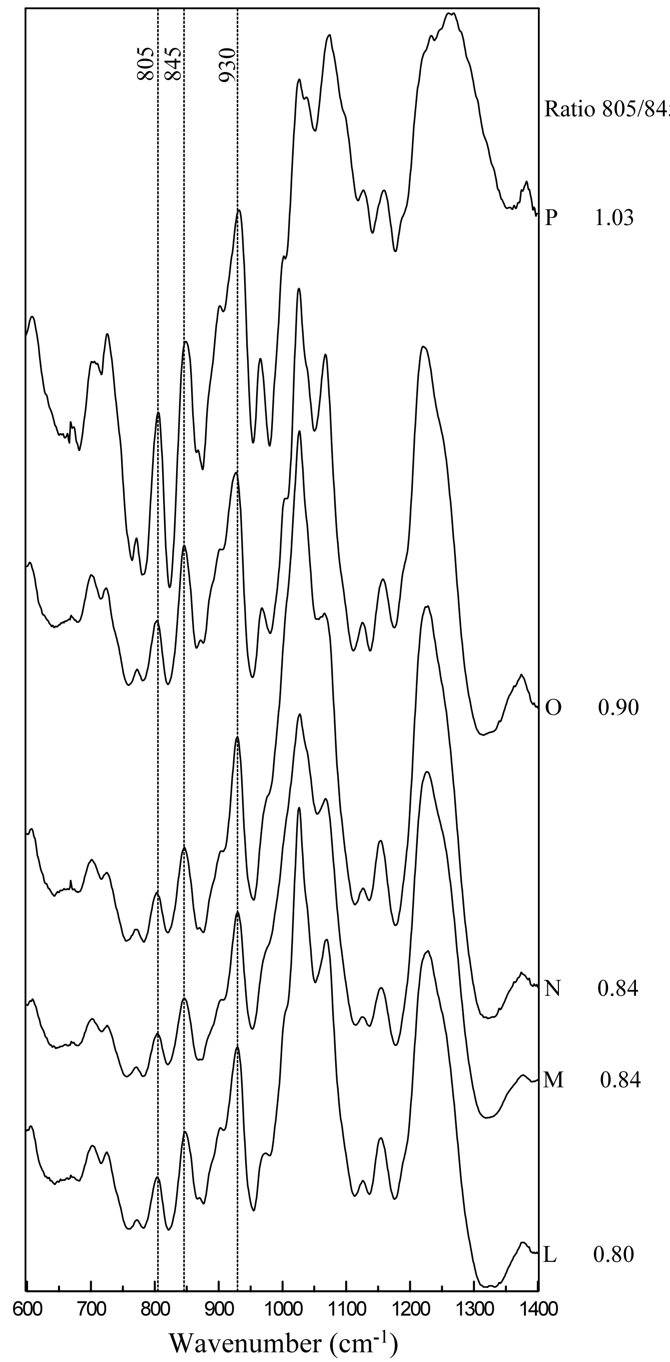

Fig. 4. FTIR spectra of alkali treated carrageenan: (L) *C. jubata* (non-fertile thalli), (M) *C. jubata* (tetrasporophytes), (N) *C. jubata* (female gametophytes), (O) *G. crenulatus* (tetrasproblastic thalli), (P) *E. denticulatum* (sample from Sigma, C-4014).

The ι or ι (κ) types were found in species of Phyllophoraceae and Solieriaceae families. Seasonal variations were found in the carrageenans from *A. devoniensis* and *G. crenulatus*. Following the analysis of the FTIR spectra, these carrageenophytes have shown to present a variation in the amount of 3,6-anhydro-D-galactose-2-sulphate in the spring/summer and autumn/winter. The ratio 805/845 (Figs. 3 and 4) in the autumn/winter samples is greater than that was found in spring/summer ones, indicating a bigger percentage of ι-type. However, for definitive conclusions, it is necessary to analyse more samples by ^{13}C NMR.

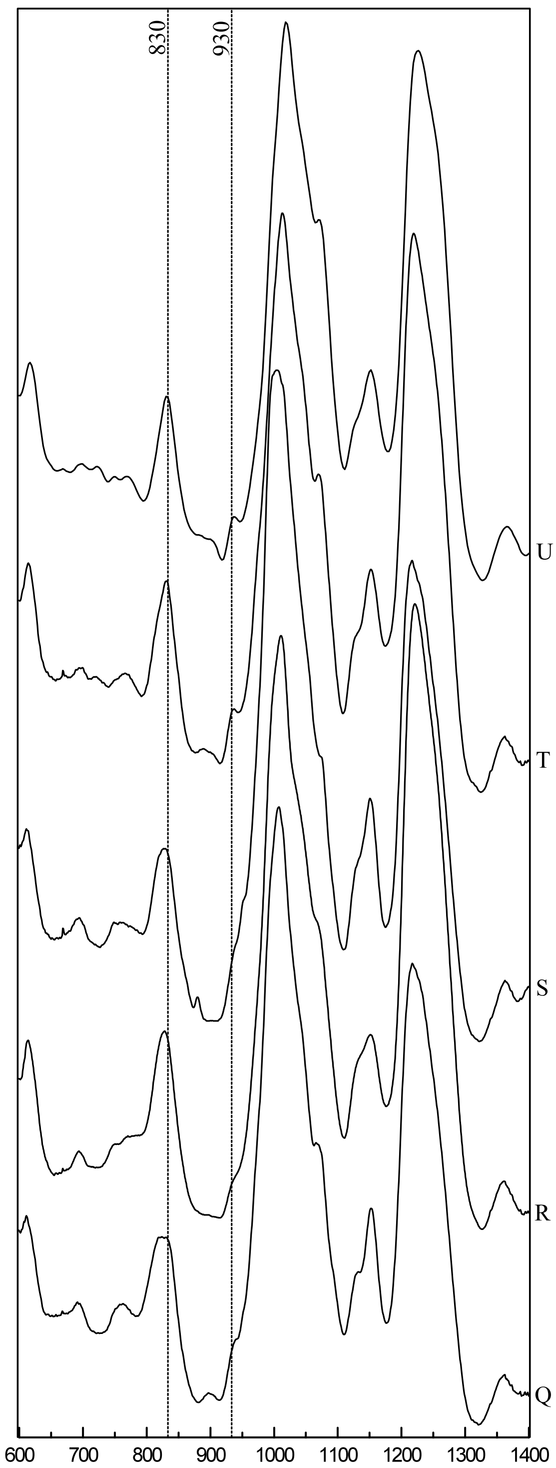

Fig. 5. FTIR spectra of alkali treated carrageenan: (Q) *C. crispus* (tetrasporophytes), (R) *G. pistillata* (heterosporic thalli), (S) *G. pistillata* (tetrasporophytes), (T) *C. acicularis* (tetrasporophytes), (U) *C. teedei* var. *lusitanicus* (tetrasporophytes).

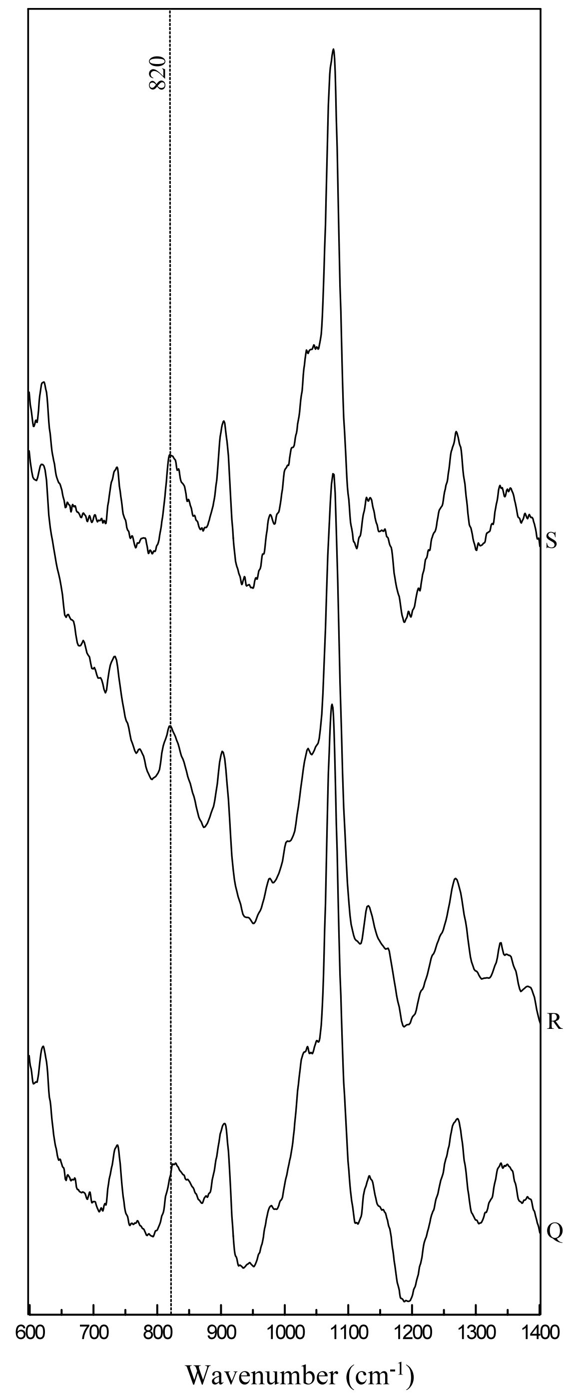

Fig. 6. FT-Raman spectra of alkali treated carrageenan: (Q) *C. crispus* (tetrasporophytes), (R) *G. pistillata* (heterosporic thalli), (S) *G. pistillata* (tetrasporophytes).

5. Conclusions

This study shows that several carrageenophytes of the Portuguese coast could be used for industrial applications. So, κ and λ fractions can be provided, respec-

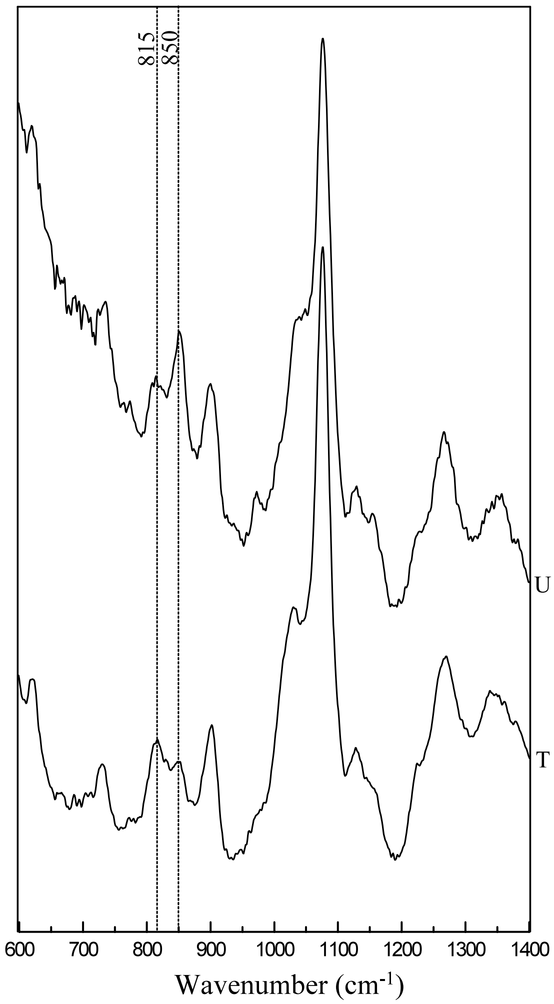

Fig. 7. FT-Raman spectra of alkali treated carrageenan: (T) *C. acicularis* (tetrasporophytes), (U) *C. teedei* var. *lusitanicus* (tetrasporophytes).

tively, by gametophytes and tetrasporophytes of Petrocelidaceae and Gigartinaceae species. The ι fraction could be obtained from the Phyllophoraceae and Cystocloniaceae species, in substitution of traditional ι-carrageenan sources (*E. denticulatum*).

The presence of significant populations of *C. teedei* var. *lusitanicus*, producing κ–ι hybrid carrageenan will be able to constitute an important source of hybrid carrageenans, this being important regarding the increasing search of these hybrid phycocolloid in food industry of milk derivatives.

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