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# Use of FTIR, FT-Raman and $^{13}$C-NMR spectroscopy for identification of some seaweed phycocolloids

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**Abstract**

Many seaweeds produce phycocolloids, stored in the cell wall. Members of the Rhodophyceae produce polysaccharides the main components of which are galactose (galactans)—agar and carrageenan. In addition, alginic acid is extracted from members of the Phaeophyceae. This is a binary polyuronide made up of mannuronic acid and guluronic acid. The wide uses of these phycocolloids are based on their gelling, viscosifying and emulsifying properties, which generate an increasing commercial and scientific interest. In this work, the FTIR and FT-RAMAN spectra of carrageenan and agar, obtained by alkaline extraction from different seaweeds (e.g. *Mastocarpus stellatus*, *Chondrus crispus*, *Calliblepharis jubata*, *Chondracanthus acicularis*, *Chondracanthus teedei* and *Gracilaria gracilis*), were recorded in order to identify the type of phycocolloid produced. The spectra of commercial carrageenan, alginic acid and agar samples (SIGMA and TAAB laboratories) were used as references. Special emphasis was given to the 500–1500 cm$^{-1}$ region, which presents several vibrational modes, sensitive to the type of polysaccharide and to the type of glycosidic linkage. The FT-Raman spectra present a higher resolution than FTIR spectra, this allowing the identification of a larger number of characteristic bands. In some cases, phycocolloids can be identified by FT-Raman spectroscopy alone.


*Keywords:* FTIR; FT-Raman; $^{13}$C-NMR; Carrageenan; Agar; Alginate

## 1. Introduction

Phycocolloids (e.g. carrageenan, agar and alginate) are special polysaccharides produced by several seaweed species. Carrageenan and agar are sulfated polysaccharides extracted from some Rhodophyceae. The principal feature distinguishing the highly sulfated carrageenans from the less sulfated agars is the presence of D-galactose and anhydro-D-galactose in the former and D-galactose, L-galactose, or anhydro-L-galactose in the latter [1]. Alginic acid, made up of blocks of mannuronic and guluronic acid, is extracted from some members of Phaeophyceae. The extraction process is based on the conversion of an insoluble mixture of alginic acid salts of the cell wall, in a soluble salt (alginate) which is appropriate for the water extraction [2]. These phycocolloids have a range of different and special properties, with wide uses, which is generating an increasing commercial and scientific interest.

In order to define the seaweed, carrageenan and agar nature, Anderson et al. (1968) [3], Stancioff and Stanley (1969) [4], Christiaen and Bodard (1983) [5] were the former to use Infrared Spectroscopy. Vibrational spectroscopy (Infrared and Raman) can reveal detailed information concerning the properties and structure of materials at a molecular level. Infrared spectroscopy (IR) was until recently the most widely used vibrational technique for studying natural products. This spectroscopic method presents three main advantages: it is fast, non-destructive, and it demands small sample quantities [6].

In contrast, the application of traditional Raman spectroscopy was limited until recently, due to the laser-induced fluorescence (strong background signal which is detected when some samples, such as biological bio-

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chemical compounds, are excited with visible lasers) and risk of sample destruction by light energy. Moreover, recording the spectra with good signal-to-noise ratio was often time-consuming. These limitations were greatly overcome through the development of near-IR Fourier transform Raman spectroscopy (FT-Raman), with which fluorescence, risk of sample destruction and spectra recording time are greatly reduced [7] while the spectral resolution and signal-to-noise ratio are enhanced.

$^{13}$C-NMR spectroscopy is used (e.g.) to quantify the κ–ι ratio in a carrageenan blend. By combining $^{13}$C-NMR with FTIR, the carrageenan nature can be established [8]. Solutions of λ-carrageenan and its variants are extremely viscous; hence it has not been possible to get a good NMR spectrum in liquid phase studies [9].

In this work, the three spectroscopic methods (FTIR, FT-Raman and $^{13}$C-NMR) were combined in order to analyze the phycocolloid samples extracted from *Mastocarpus stellatus* (Petrocelidaceae), *Chondrus crispus* (Gigartinaceae), *Calliblepharis jubata* (Cystocloniaceae), *Chondracanthus acicularis* (Gigartinaceae), *Chondracanthus teedei* (Gigartinaceae) and *Gracilaria gracilis* (Gracilariaceae). The identification of these phycocolloids was made by the comparison of the recorded spectra with those obtained from commercially available samples.

## 2. Materials and methods

Samples of *M. stellatus* (Petrocelidaceae), *C. crispus* (Gigartinaceae), *C. jubata* (Cystocloniaceae), *C. acicularis* (Gigartinaceae) *C. teedei* (Gigartinaceae) and *G. gracilis* (Gracilariaceae) were collected from Buarcos bay (Figueira da Foz, Portugal), washed with distilled water to eliminate residues from the thalli surface, and dried in a oven at 60 °C.

For carrageenan and agar extraction, 1 g of dry sample was rehydrated for 12 h, in order to eliminate the hydro-soluble fraction, and immersed in a methanol–acetone (1:1) mixture, for the elimination of the organic-soluble fraction [10]. The carrageenophytes were placed in 150 ml NaOH (1 M) at 80 °C for 3 h (alkali treatment) and the pH adjusted to 8. Hot filtration was performed, under suction, twice and the phycocolloid was precipitated adding ethanol (96%). The agarophyte was placed in 150 ml distilled water at 90 °C for 4 h (adjustment of pH to 6–8). A hot filtration under suction was performed twice. Agar was extracted using a process of freeze/thawing. The water fraction was removed and the remaining refrozen (this step was repeated to remove all of the water).

Standard samples were obtained from Sigma (κ-carrageenan, type III, C-1263; λ-carrageenan, type IV, C-3889; ι-carrageenan, type V, C-4014; alginic acid, A0682) and TAAB Laboratories (agar, A010).

The FTIR spectra were recorded on an IFS 55 spectrometer, using a Golden Gate single reflection 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 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). It should be noted that our chemical shifts values are larger (2.5 ppm) than those reported by Usov [11] and Zinoun [12].

## 3. Results

### 3.1. Carrageenan analysis by FT-Raman and FTIR

The FTIR and FT-Raman spectra of κ-carrageenan and of *M. stellatus* (female gametophyte) are shown in Fig. 1. The spectra show a band at approximately 845

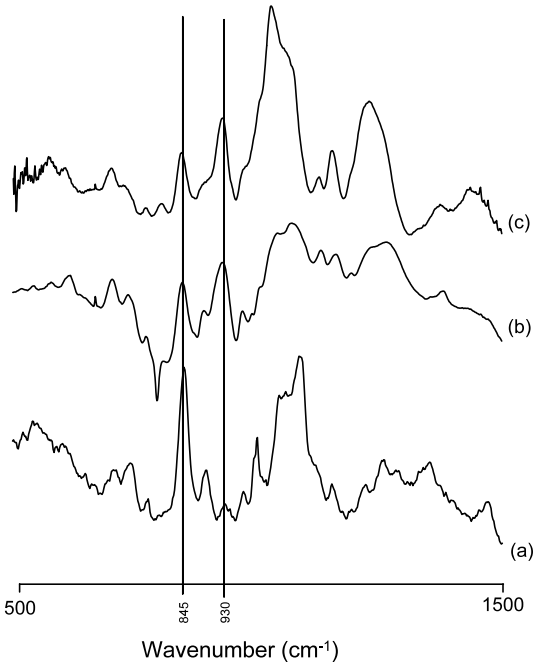


Fig. 1. FT-Raman (a) and FTIR (b) spectra of commercial κ-carrageenan and FTIR (c) spectra of *M. stellatus* (female gametophytes) extracted carrageenan.

$cm^{-1}$, which is assigned to D-galactose-4-sulfphate. The presence of a strong band at approximately 930 $cm^{-1}$ in the FTIR spectrum, weak in FT-Raman spectrum, indicates the presence of 3,6-anhydro-D-galactose in both samples [13–15].

Fig. 2 presents the FTIR and FT-Raman spectra of ι-carrageenan and of the phycocolloids extracted from *C. jubata* (non-fertile and female gametophyte). The spectra of these samples also show the bands at approximately 930 and 845 $cm^{-1}$. A new feature appears at approximately 805 $cm^{-1}$, indicating the presence of two sulfate ester groups on the anhydro-D-galactose residues, a characteristic of this type of carrageenan [10,13].

The FTIR and FT-Raman spectra of λ-carrageenan and of *C. crispus* tetrasporophytes are shown in Fig. 3. These samples present high sulfate content as indicated by the broad band between 820 and 830 $cm^{-1}$ [13–15].

A comparative study of the vibrational spectra of carrageenan from *C. crispus* and *C. acicularis* tetrasporophytes is shown in Fig. 4. The FTIR and FT-Raman spectra show characteristic peaks of λ-family carrageenan [14]. The *C. crispus* carrageenan FTIR spectrum (Fig. 4b) shows a broad peak in approximately 820–830 $cm^{-1}$ and *C. acicularis* (Fig. 4d) shows a sharp peak. Between 815 and 900 $cm^{-1}$, FT-Raman spectrum shows two peaks (825 and 900 $cm^{-1}$) in *C. crispus* (Fig. 4a) and three peaks (815, 850 and 900 $cm^{-1}$) in *C. acicularis* (Fig. 4c).

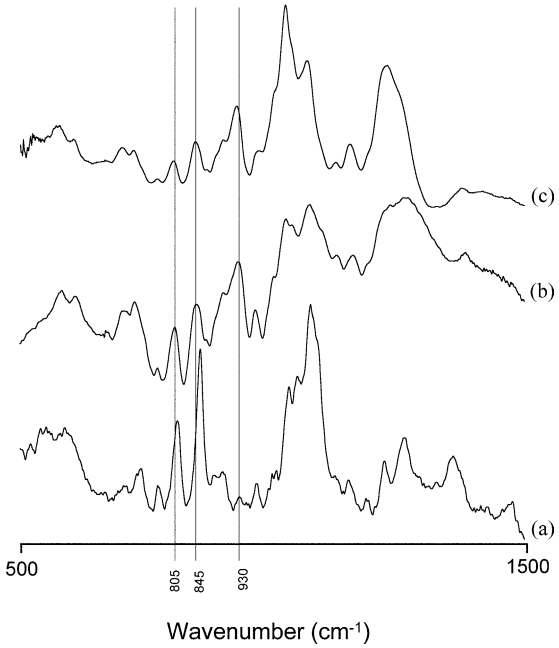


Fig. 2. FT-Raman (a) and FTIR (b) spectra of commercial ι-carrageenan and FTIR (c) spectra of *C. jubata* (non-fertile thalli) extracted carrageenan.

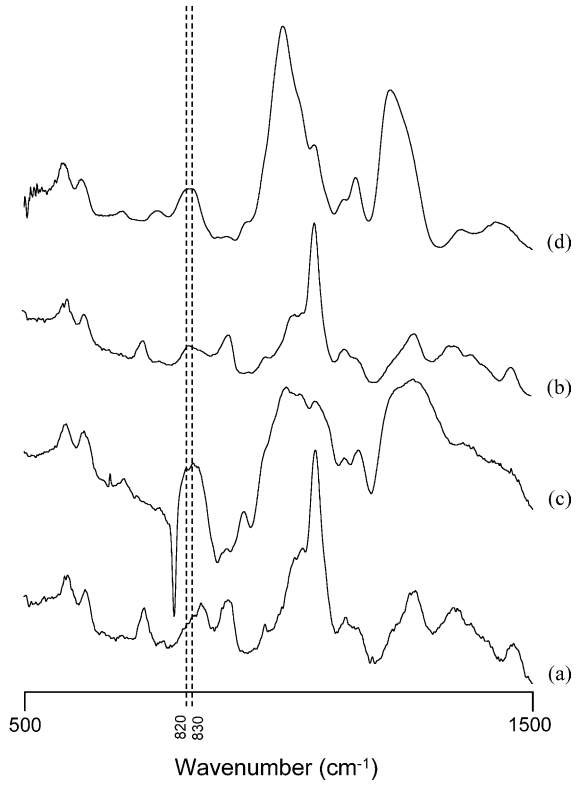


Fig. 3. FT-Raman (a) and FTIR (b) spectra of commercial λ-carrageenan, FT-Raman (c) and FTIR (d) spectra of *C. crispus* (tetrasporophytes) extracted carrageenan.

### 3.2. Carrageenan analysis by $^{13}$C-NMR

Table 1 presents the chemical shifts for κ, ι (commercial samples) and κ–ι hybrid (sample from *C. teedei*) carrageenans.

### 3.3. Agar analysis by FT-Raman and FTIR

Fig. 5 shows the FTIR and FT-Raman spectra of agar obtained commercially and extracted from *G. gracilis* seaweeds. The bands at 740 and 770 $cm^{-1}$ are both strong in the FT-Raman spectra and weak in the FTIR one, and are assigned to the skeletal bending of the galactose ring. In addition, the FT-Raman spectrum shows a strong band centered at approximately 837 $cm^{-1}$, which is absent in the FTIR spectra. This band is associated with the CH vibration coupled with C–OH related modes in methyl 3,6-anhydro-D-galactose residues with a α-configuration [7]. Moreover, the spectral feature at approximately 890 $cm^{-1}$ is also particularly intense in the FT-Raman spectrum of agar and is mainly associated with the C–H bending at the anomeric carbon in β-galactose residues [7]. As stated previously, the presence of a strong band in the FTIR spectra at 930 $cm^{-1}$ and weak in the FT-Raman spectrum is indicative of the occurrence of 3,6-anhydro-D-galactose.

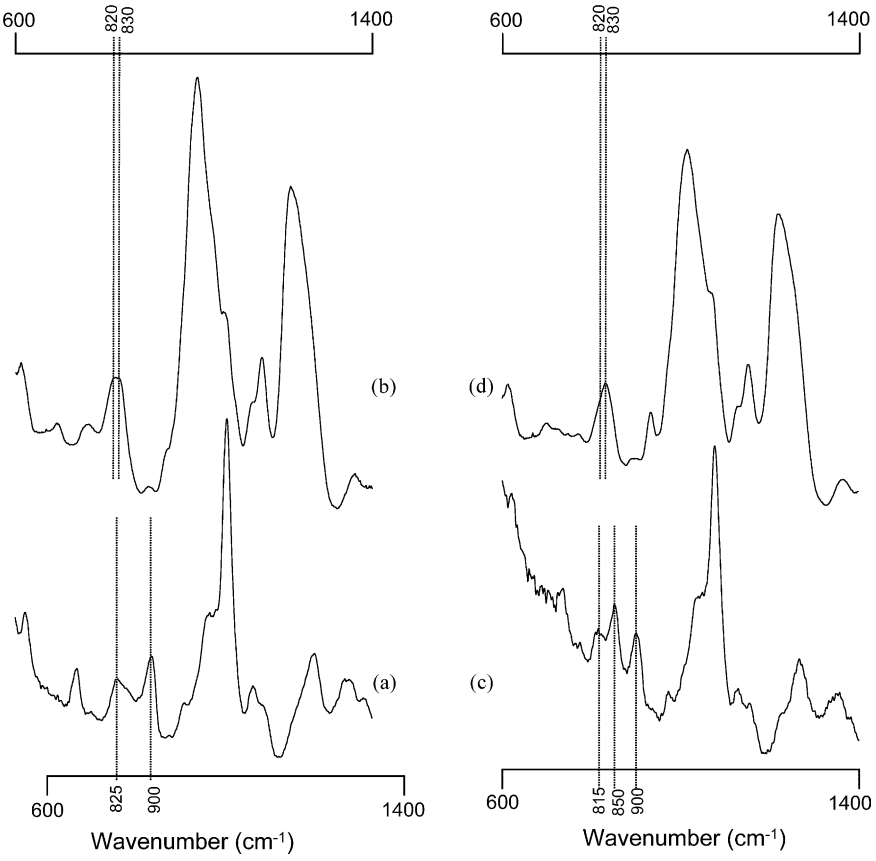


Fig. 4. FT-Raman (a) and FTIR (b) spectra of carrageenan extracted from *C. crispus* (tetrasporophytes); FT-Raman (c) and FTIR (d) spectra of carrageenan extracted from *C. acicularis* (tetrasporophytes).

The most prominent band in the FT-Raman spectra of agar, centered at approximately 1079 $cm^{-1}$, has contributions from several vibrational modes, namely to the C–O and C–C stretchings and to the C–C–O and C–O–H deformations [6,7].

### 3.4. Alginates analysis by FT-Raman and FTIR

Fig. 6 presents the FTIR and FT-Raman spectra of commercial alginate. The most prominent FT-Raman band centered at 950 $cm^{-1}$ is mainly due to the O–H deformation mode, while the band at 1400 $cm^{-1}$, strong in both FTIR and FT-Raman spectra, is ascribed to the deformation of the $CH_2$ groups. The C–O–C and C–OH stretching modes give rise to several close-lying bands in the spectral regions of 1250–1290 and 1000–1025 $cm^{-1}$, respectively [16,17].

## 4. Discussion

### 4.1. Carrageenan analysis by FT-Raman and FTIR

The study of carrageenans by FTIR and FT-Raman spectroscopy (Figs. 1–4) shows the presence of very strong absorption bands in the 1210–1260 $cm^{-1}$ region

Table 1
$^{13}$C-NMR chemical shifts for κ, ι (commercial samples) and κ–ι carrageenan (from *C. teedei*)

| Carrageenan type | Residue | $C_1$ | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ |
|---|---|---|---|---|---|---|---|
| κ | κG | 104.7 | 71.8 | 81.4 | 76.4 | 77.0 | 63.5 |
| | κA | 97.4 | 72.1 | 81.4 | 80.6 | 79.1 | 71.8 |
| ι | ιG | 104.4 | 71.6 | 79.3 | 74.4 | 77.2 | 63.5 |
| | ιA | 94.3 | 77.2 | 80.0 | 80.5 | 79.3 | 71.6 |
| κ–ι | κG | 104.7 | 71.9 | 81.4 | 76.4 | 77.0 | 63.5 |
| | κA | 97.5 | 71.9 | 81.4 | 80.6 | 79.1 | – |
| | ιG | 104.7 | – | 79.1 | 74.4 | 77.0 | 63.5 |
| | ιA | 94.3 | 77.0 | – | 80.6 | – | 71.9 |

G, galactose; A, anhydrogalactose.

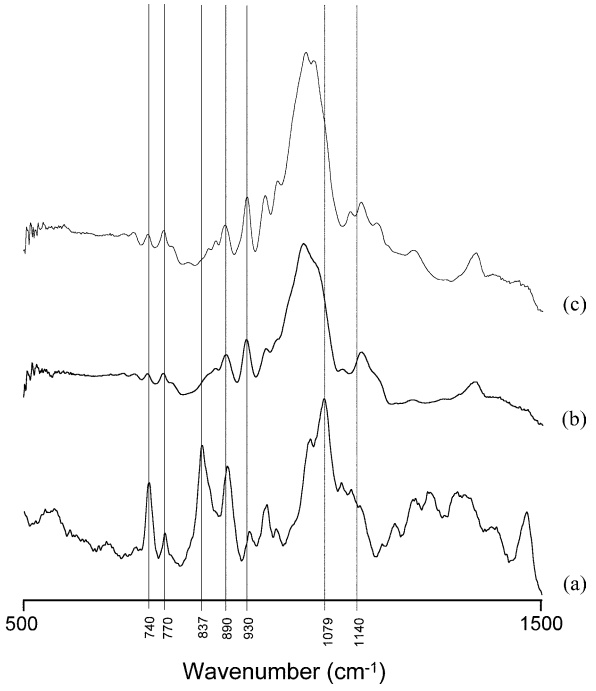


Fig. 5. FT-Raman (a) and FTIR (b) spectra of commercial agar, FTIR (c) spectra of *G. gracilis* extracted agar.

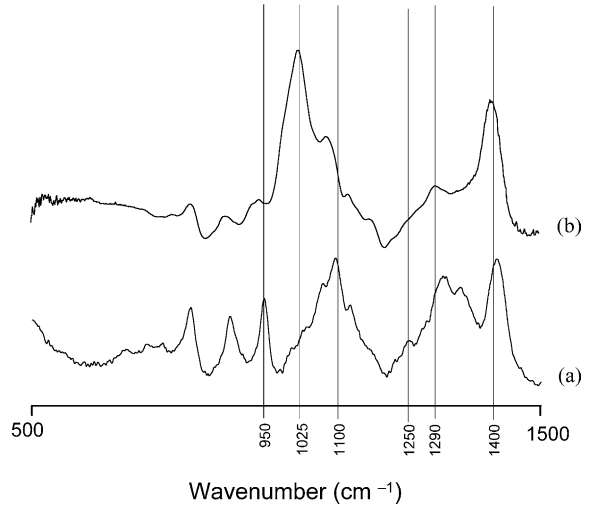


Fig. 6. FT-Raman (a) and FTIR (b) spectra of commercial alginate.

(S=O of sulfate esters) and in 1010–1080 $cm^{-1}$ region (glycosidic linkage) in all carrageenan types. The other chemical groups are characteristics of a given carrageenan type: 3,6-anhydro-D-galactose at 925–935 $cm^{-1}$, D-galactose-4-sulfate at 840–850 $cm^{-1}$, D-galactose-2-sulfate at 820–830 $cm^{-1}$, D-galactose-6-sulfate at 810–820 $cm^{-1}$, and 3,6-anhydro-D-galactose-2-sulfate at 800–805 $cm^{-1}$ [18].

In the FTIR spectra, both κ and ι-carrageenan present the 845–850 $cm^{-1}$ band, but 800–805 $cm^{-1}$ band is characteristic and distinctive of ι-carrageenan. The relative shape of the 820–830 $cm^{-1}$ band allows us to distinguish the λ (broad band) and ξ-variant (sharp band) [14,15]. In comparative studies of carrageenan types, the FTIR spectra provide enough information. However, FT-Raman is a more easily applied method and the correspondent spectra have a clear resolution. Discrimination between κ- and ι-carrageenan is based in the 805 $cm^{-1}$ peak, which has a stronger signal in FT-Raman spectra than in FTIR one.

FT-Raman spectra have an 815–900 $cm^{-1}$ band with additional information to distinguish the λ-family carrageenan variants when compared with FTIR spectra. The λ-variant spectrum shows the 825 and 900 $cm^{-1}$ peaks, and ξ-variant spectrum shows the 815, 850 and 900 $cm^{-1}$ peaks. This may be an advantage of FT-Raman spectroscopy when compared with FTIR.

### 4.2. Carrageenan analysis by $^{13}$C-NMR

$^{13}$C-NMR spectroscopy provides information on the environment of every carbon atom in the molecule [12,19]. The anomeric carbon signals at 104.7 and 97.4 ppm are characteristic of κ-carrageenan. The anomeric signals at 104.4 and 94.3 ppm are characteristic of ι-carrageenan. The hybrid κ–ι carrageenan presents three peaks 104.7, 97.5 and 94.3 ppm in the anomeric carbon zone. Is not possible get a good NMR spectrum in liquid phase studies with solutions of λ-carrageenan, because of its high viscosity [19].

### 4.3. Agar analysis by FT-Raman and FTIR

The spectrum of *G. gracilis* is very similar to those of the agar (sample from TAAB Lab., A010) in the region 500–1500 $cm^{-1}$. Both show the characteristic bands at 740, 770, 837 $cm^{-1}$ (FT-Raman) and 890 $cm^{-1}$ (FT-Raman and FTIR) [7].

According to Christiaen and Bodard [5] the 890 $cm^{-1}$ band is specific for agar and can be attributed to anomeric C–H of β-galactose residues [7].

There is a small band of 895–900 $cm^{-1}$ that is generally absent in the carrageenan spectra and is visible in the agar spectra. This permits the distinction between these phycocolloids [18].

### 4.4. Alginates analysis by FT-Raman and FTIR

As stated previously, different types of alginate present different proportions and/or different alternating patterns of guluronic and mannuronic units. The presence of these acids can be identified from their characteristic bands: while the guluronic units originate a band at approximately 1025 $cm^{-1}$, the mannuronic units originate a band at approximately 1100 $cm^{-1}$. Thus, the guluronic/mannuronic concentration ratio,

characterizing a certain alginate sample, can be inferred from the relative intensity ratio of the 1025 and 1100 $cm^{-1}$ bands [16].

## 5. Conclusions

Precise assignment of the bands in both FTIR and FT-Raman spectra of polysaccharides in general is not an easy task, as most of the vibrations arise from highly coupled C–O, C–OH and C–C groups. However, the present study allows us to identify some bands, which are characteristic of each phycocolloid (carrageenan, agar and alginate) studied.

The present study shows that FT-Raman spectroscopy allows an easier and more accurate monitoring of agar than FTIR spectroscopy. In fact, the FT-Raman spectra of agar samples show a higher resolution and better definition of bands than the FTIR spectra. Important characteristic bands, such those at 740, 770 and 837 $cm^{-1}$, are easily observed in the FT-Raman and correspond to extremely weak absorptions in the infrared. Variants of the λ-family carrageenans can be more clearly identified by FT-Raman.

Until now, the information of FT-Raman spectrum has been considered a complementary spectroscopic technique in the identification of phycocolloid identity [7]. However, according to our results we can conclude that carrageenans (except hybrids), agar and alginate can be identified by FT-Raman spectroscopy only. Moreover, a quantitative analysis based on the vibrational spectra is possible, and the preparation of a good calibration set deserves further attention.

In this work, the characterization of seaweed phycocolloids has been complemented by $^{13}C$-NMR spectroscopy, especially for distinction of hybrid carrageenans.

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