Food Hydrocolloids xxx (2009) 1–7

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman)

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ARTICLE INFO

Article history:
Received 11 June 2008
Accepted 24 November 2008

Keywords:
Polysaccharides
Seaweed
Phycocolloids
Carrageenan
FTIR-ATR
FT-Raman

ABSTRACT

The wide industrial application of phycocolloids (e.g. alginates, agar and carrageenans) is based on their particular properties to form gels in aqueous solution. These seaweed polysaccharides present a chemical structure related with the taxonomic position of the algae: carrageenans are produced by carrageenophytes (red algae belonging mainly to the genera *Kappaphycus*, *Eucheuma*, *Chondrus*, *Gigartina* and *Chondracanthus*). Recently, new spectroscopic techniques have provided more accurate identification of the natural composition of the polysaccharides produced by these seaweeds. With the combination of two spectroscopic techniques (FTIR-ATR and FT-Raman) it is possible to identify the principal seaweed colloids in ground seaweed samples as in extracted material. Since the seaweed samples receive the minimum of handling and treatment (e.g. they are simply dried and ground), the composition determined represents, as accurately as possible, the native composition of the phycocolloids.

1. Introduction

Many seaweeds produce hydrocolloids, associated with the cell wall and intercellular spaces. Members of the red algae (Rhodophyta) produce galactans (e.g. carrageenans and agars) and the brown algae (Ochrophyta, Phaeophyceae) produce uronates (alginates). Carrageenans represent one of the major texturising ingredients used by the food industry; they are natural ingredients, which have been used for decades in food applications and are generally regarded as safe (GRAS).

The phycocolloid *carrageenin*, as it was first called, was discovered by the British pharmacist Stanford in 1862 who extracted it from Irish moss (*Chondrus crispus*). The name was later changed to carrageenan so as to comply with the '-an' suffix for the names of polysaccharides. The modern carrageenan industry dates from the 1940s, receiving its impetus from the dairy industry where carrageenan was found to be the ideal stabilizer for the suspension of cocoa in milk chocolate.

The commercial carrageenans are normally divided into three main types: kappa-, iota- and lambda-carrageenan. The idealized disaccharide repeating units of these carrageenans are given in Fig. 1. Generally, seaweeds do not produce these idealized and pure carrageenans, but more likely a range of hybrid structures. Several other carrageenan repeating units exist: e.g. xi, theta, beta, mu and nu (Fig. 1). The precursors (mu and nu), when exposed to alkali conditions, are modified into kappa and iota, respectively, through formation of the 3,6-anhydrogalactose bridge (Rudolph, 2000).

Infrared (IR) spectroscopy was, until recently the most frequently used vibrational technique for the study of the chemical composition of phycocolloids. This technique presents two main advantages: it requires minute amounts of sample (milligrams), and it is a non-aggressive method with reliable accuracy (Pereira, Sousa, Coelho, Amado, & Ribeiro-Claro, 2003). However, conventional IR spectroscopy requires laborious procedures to obtain spectra with a good signal/noise ratio (Chopin & Whalen, 1993). This limitation was overcome with the development of interferometric IR techniques (associated with the Fourier transform algorithm), known as FTIR spectroscopy (Fourier transform IR). More recently, Pereira and collaborators had used a technique of analysis on the basis of FTIR-ATR (from attenuated total reflectance) spectroscopy, allowing for the determination of the composition of the

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doi:10.1016/j.foodhyd.2008.11.014

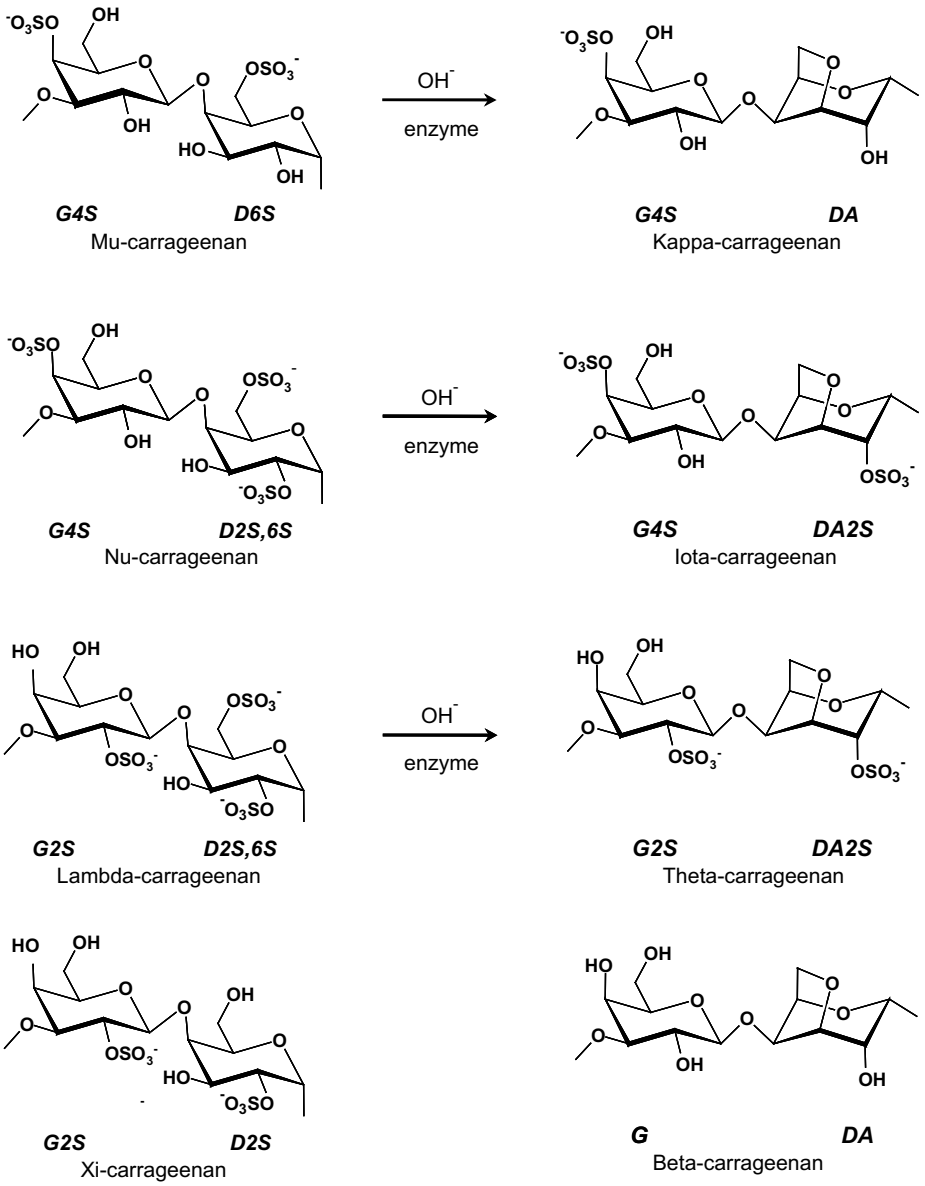

Fig. 1. Idealized units of the main types of carrageenan.

different phycocolloids from dried ground seaweed, without having to prepare tablets of KBr (Pereira, 2006; Pereira & Mesquita, 2004).

In contrast to FTIR, the application of conventional Raman spectroscopy was limited until recently, due to need for an incident visible laser in dispersive spectrometers: the visible laser light often excites electronic transitions in biochemical samples, which can lead to either sample degradation or strong background signal from unwanted laser-induced fluorescence. The use of Nd:YAG lasers operating at 1064 nm (far from the visible region) in interferometric spectrometers has been generalized to decrease the fluorescence level and avoid sample degradation. The modern FT-Raman spectrometers have been used to produce good quality Raman spectra from seaweed samples (Dyrby et al., 2004; Matsuhiro, 1996; Pereira et al., 2003).

In this work, a combined FTIR-ATR and FT-Raman spectroscopy study was used to identify the principal phycocolloids, namely, alginate, kappa-, mu-, iota-, nu-, lambda-, theta- and xi-carrageenan. Since the analysis of ground seaweed samples required minimal treatment (the seaweeds are simply dried and ground), the determined composition represents, as accurately as possible, the natural colloid composition of each seaweed.

2. Materials and methods

2.1. Algal material and standard samples of hydrocolloids

Specimens of red algae (Rhodophyta) *Kappaphycus alvarezii* (Gigartinales), *Eucheuma denticulatum* (Gigartinales), *Betaphycus* gelatinum (Gigartinales), *C. crispus* (cultivated strain, Gigartinales), were collected respectively in Tanzania, Madagascar, Philippines and Canada. Specimens of red algae (Rhodophyta), *Calliblepharis jubata* (Gigartinales), *Chondracanthus teedei* and *Chondracanthus teedei* var. *lusitanicus* (Gigartinales), *C. crispus* (Gigartinales) were collected in the central zone of the western coast of Portugal.

Standard samples were obtained from Sigma (kappa-carrageenan, type III, C-1263; iota-carrageenan, type V, C-4014 and CP Kelco (pure lambda-carrageenan).

The sample composition and purity were controlled by NMR.

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Table 1
Phycocolloid composition determined by NMR.

Species	Lifecycle phase	Phycocolloid composition
		Alkali-extracted[a] (%mol)
Red algae (Rhodophyta)		
Chondracanthus teedei	Non-fructified	50.0 κ, 50.0 ι G4S-DA, G4S-DA2S
Chondracanthus teedei var. *lusitanicus*	Tetrasporophyte	67.0 ξ, 33.0 θ G2S-D2S, G2S-DA2S
Calliblepharis jubata	Non-fructified	100.0 ι G4S-DA2S
Eucheuma denticulatum	–	4.0 κ, 96.0 ι G4S-DA, G4S-DA2S
Kappaphycus alvarezii	–	93.0 κ, 7.0 ι G4S-DA, G4S-DA2S
Betaphycus gelatinum	–	50.0 κ, 50.0 β G4S-DA, G-DA

[a] Composition determined by ^{1}H-NMR, except the analysis of *C. teedei* (tetrasporophyte) carrageenan, made by ^{13}C-NMR; the carrageenans are identified according to the Greek lettering system and letter code proposed by Knutsen et al. (1994).

2.2. Preparation of ground seaweed samples for FTIR-ATR and FT-Raman

The seaweed samples were rinsed in distilled freshwater to eliminate salt and debris from the thallus surface and dried to constant weight at 60 °C. The dried seaweeds were finely ground in order to render the samples uniform. For FTIR analysis the samples do not need additional treatment. The analysis by FT-Raman requires that these are without pigmentation. The lack of pigmentation can be achieved by sun drying (process used by collectors/producers of commercial seaweeds) or by pigment elimination in the laboratory by the addition of calcium hypochlorite solution (4%, 30-60 s, 4 °C) (Pereira, 2004).

2.3. Phycocolloid extraction

Before phycocolloid extraction, the ground dry material was rehydrated and pre-treated with a moisture of methanol 100% and acetone 100% to eliminate the organosoluble fraction (Zinoun & Cosson, 1996).

For extraction of the native phycocolloid, the seaweed samples were placed in distilled water (50 mL/g), pH 7 at 85 °C for 3 h. For an alkaline extraction (resembling the industrial method), the samples were placed in a solution (150 mL/g) of NaOH (1 M) at 80–85 °C for 3–4 h according to Pereira (2006), and neutralised to pH 6–8 with HCl (0.3 M).

The solutions were hot filtered, twice, under vacuum, through cloth and glass fibre filter. The extract was evaporated under vacuum to one-third of the initial volume. The carrageenan was precipitated by adding the warm solution to twice its volume of ethanol (96%). Coagula were dried in an oven for 48 h at 60 °C, and then weighed to determine the phycocolloid content (Pereira & Mesquita, 2004).

2.4. FTIR-ATR and FT-Raman analysis

Samples were softly milled to ensure homogeneity. Both ATR and Raman require no more than 2 mm^3 of sample (to cover the ATR diamond window or to focus the laser beam).

The FTIR spectra of sample materials (ground dried seaweed, native and alkali-modified carrageenan) 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 independent measurements with 128 scans each at a resolution of 2 cm^{-1}.

The corresponding FT-Raman spectra were recorded on an RFS-100 Bruker FT-spectrometer using an Nd:YAG laser with an excitation wavelength of 1064 nm. Each spectrum was the average of two repeated measurements, with 150 scans at a resolution of 2 cm^{-1}.

2.5. NMR analysis

^{1}H-NMR spectra were taken on a Bruker AMX600 spectrometer operating at 500.13 MHz at 65 °C. Typically 64 scans were taken with an interpulse delay of 5 s (T_1 values for the resonance of the anomeric protons of kappa- and iota-carrageenan are shorter than 1.5 s). Sample preparation for the ^{1}H-NMR experiments involved dissolving the carrageenan sample (5 mg/mL) at 80 °C in D_2O containing 1 mM TSP (3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt) and 20 mM Na_2HPO_4, followed by sonication for three times 1 h in a sonicator bath (Branson 2510) and aliquots of the sonicated solutions were transferred to NMR tubes and analysed, according to Pereira, van de Velde, and Mesquita (2007). Chemical shifts (δ) are referred to internal TSP standard ($\delta = -0.017$ ppm) relative to the IUPAC recommended standard DSS for ^{1}H according to van de Velde, Pereira, and Rollema (2004). Assignments of the ^{1}H-NMR spectra were based on the chemical shift data summarized by van de Velde and de Ruiter (2002) and van de Velde et al. (2004).

3. Results and discussion

The main results of the analyses are listed in Table 2 (FTIR-ATR) and Table 3 (FT-Raman). The assignments of the IR spectra were mostly based on the previous work of Chopin and Whalen (1993)

Table 2
Identification of carrageenan types by infrared spectroscopy (adapted from Chopin & Whalen, 1993; Pereira, 2006).

Wavenumbers (cm^{-1})	Bond(s)/group(s)	Letter code	Type of carrageenan							
			Kappa (κ)	Mu (μ)	Iota (ι)	Nu (ν)	Beta (β)	Theta (θ)	Lambda (λ)	Xi (ξ)
1240–1260	S=O of sulphate esters		+	++	++	+++	−	++	+++	++
1070	C–O of 3,6-anhydrogalactose	DA	+	−	+	−	+	+	−	−
970–975	Galactose	G/D	+	s	+	s	+	+	−	−
930	C–O of 3,6-anhydrogalactose	DA	+	−	+	−	+	+	−	−
905	C–O–SO_3 on C2 of 3,6-anhydrogalactose	DA2S	−	−	+	−	−	+	−	−
890–900	Unsulphated β-D-galactose	G/D	−	−	−	−	+	−	−	−
867	C–O–SO_3 on C6 of galactose	G/D6S	−	+	−	+	−	−	+	−
845	C–O–SO_3 on C4 of galactose	G4S	+	+	+	+	−	−	−	−
825–830	C–O–SO_3 on C2 of galactose	G/D2S	−	−	−	+	−	+	+	n
815–820	C–O–SO_3 on C6 of galactose	G/D6S	−	+	−	+	−	−	+	−
805	C–O–SO_3 on C2 of 3,6-anhydrogalactose	DA2S	−	−	+	−	−	+	−	−

−, Absent; +, medium; ++, strong; +++, very strong; s, shoulder peak; n, narrow peak.

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Table 3
Identification of carrageenan types by Raman spectroscopy (adapted from Pereira, 2006).

Wavenumbers (cm^{-1})	Bond(s)/group(s)	Letter code	Type of carrageenan							
			Kappa (κ)	Mu (μ)	Iota (ι)	Nu (ν)	Beta (β)	Theta (θ)	Lambda (λ)	Xi (ξ)
1240–1260	S═O of sulphate esters		++	++	++	+++	−	++	++	++
1075–1085	C–O of 3,6-anydrogalactose	DA	+++	−	+++	−	+	+	−	−
970–975	Galactose	G/D	+	+	s	s	+	+	−	−
925–935	C–O of 3,6-anydrogalactose	DA	+	−	+	−	+	+	−	−
905–907	C–O–SO_4 on C_2 of 3,6-anydrogalactose	DA2S	−	−	+	−	−	+	+	+
890–900	Unsulphated β-D-galactose	G/D	−	−	−	−	+	−	−	−
867–871	C–O–SO_4 on C_6 of galactose	G/D6S	−	s	−	+	−	−	+	−
845–850	C–O–SO_4 on C_4 of galactose	G4S	++	+	++	+	−	−	−	+
825–830	C–O–SO_4 on C_2 of galactose	G/D2S	−	−	−	+	−	+	+	−
815–825	C–O–SO_4 on C_6 of galactose	G/D6S	−	s	−	s	−	−	+	+
804–808	C–O–SO_4 on C_2 of 3,6-anhydrogalactose	DA2S	−	−	++	−	−	+	−	−

−, Absent; +, medium; ++, strong; +++, very strong; s, shoulder peak.

and Chopin, Kerin, and Mazerolle (1999). The Raman spectra were assigned based on the IR information and on the comparison between samples of known composition, controlled by NMR spectroscopy (see Table 1).

3.1. Identification of carrageenan

The FTIR-ATR and FT-Raman spectra of *K. alvarezii* were compared with those of commercial kappa-carrageenan in Fig. 2. The spectra of the ground seaweed show the main features of commercial kappa-carrageenan: a strong Raman band at approximately 845 cm^{-1} (with moderate intensity in the IR spectrum), which is assigned to D-galactose-4-sulphate (G4S) and a relatively strong band at approximately 930 cm^{-1} in the FTIR-ATR spectra, weak in FT-Raman spectrum, indicating the presence of 3,6-anhydro-D-galactose (DA).

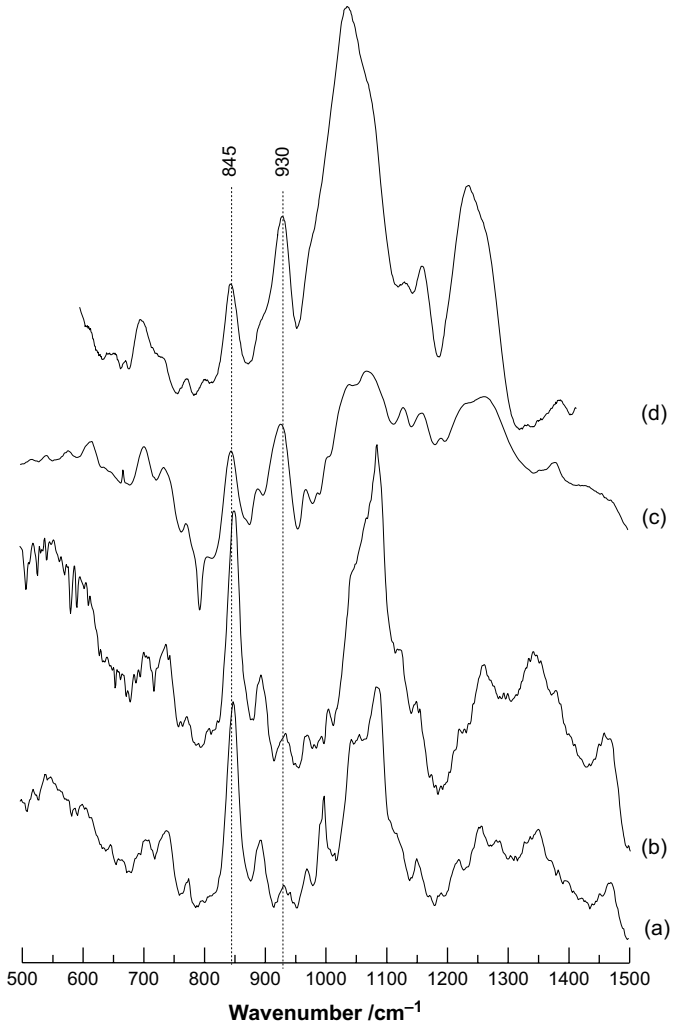

Fig. 2. Spectra of commercial kappa-carrageenan and of ground seaweed sample (*Kappaphycus alvarezii*): FT-Raman (a and b, respectively) and FTIR-ATR (c and d, respectively).

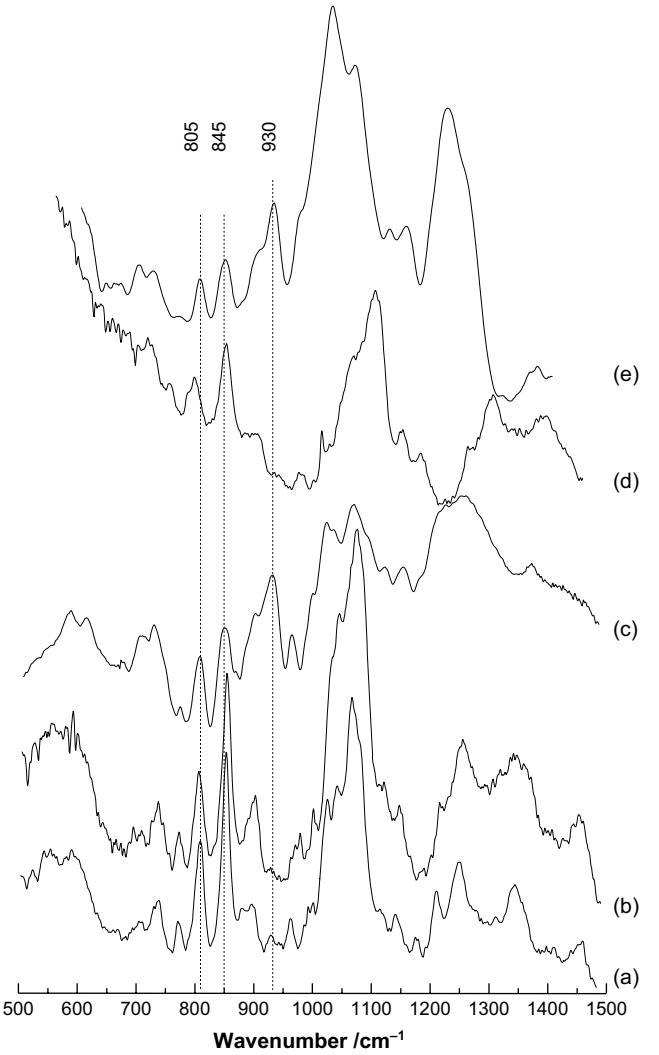

Fig. 3. FT-Raman (a) and FTIR-ATR (c) spectra of commercial iota-carrageenan; FTIR-ATR (d) spectra of ground seaweed sample (*Eucheuma denticulatum*); FT-Raman (b) and FTIR-ATR (e) spectra of ground seaweed sample *Calliblepharis jubata*, non-fructified thalli.

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Fig. 3 presents the FTIR-ATR and FT-Raman spectra of iota-carrageenan, of *C. jubata* (non-fructified thalli) and of *E. denticulatum*. The spectra of these samples also show the bands at approximately 930 and 845 cm^{-1}, with the same intensity pattern as in kappa-carrageenan. However, an additional well-defined feature is visible in both IR and Raman spectra, around 805 cm^{-1}, indicating the presence of sulphate ester in the 2-position of the anhydro-D-galactose residues (DA2S), a characteristic band of the iota-carrageenan.

Fig. 4 shows the FTIR and FT-Raman spectra of kappa/iota-hybrid carrageenan and of *C. teedei*. The spectra show two well-defined bands at 805 cm^{-1} and 845 cm^{-1}, this later being particularly intense in the FT-Raman spectra. The appearance of the two bands indicates the presence of C2-sulphated 3,6-anhydro-D-galactose and C4-sulphated galactose, respectively. The intensity ratio of the two bands in FTIR spectra can be used to infer the degree of iota/kappa-hybridisation (Correa-Diaz, Aguilar-Rosas, & Aguilar-Rosas, 1990). The presence of 3,6-anhydro-D-galactose in the sample is confirmed by the occurrence of a strong absorption band with intensity maximum at 930 cm^{-1}.

Fig. 5 illustrates the relevant contribution of Raman spectroscopy in the identification of seaweed phycocolloids. The figure presents the FTIR-ATR and FT-Raman spectra of ground seaweed and of kappa/beta-hybrid carrageenan extracted from *B. gelatinum*, the main industrial source of this type of colloid. The FTIR spectra of these samples present moderate absorption bands at 845 cm^{-1} and 890 cm^{-1}. Both bands are much more intense in the corresponding FT-Raman spectra. The lower frequency band indicates the presence of galactose units sulphated at the C-4 position, while the other band is related to the presence of non-sulphated galactose units. The intensity ratio of these two bands in FTIR spectra was calculated and used as a reliable measure of the degree of kappa/beta-hybridisation. A ratio of approximately 1 was obtained in both samples, indicating the presence of a hybrid carrageenan with identical percentage of kappa and beta fractions. In addition, the analysis of the ground seaweed by FT-Raman reveals that the biological precursors, mu- and gamma-carrageenan are absent or residual, as inferred from the absence of bands at 815 cm^{-1} and 869 cm^{-1}.

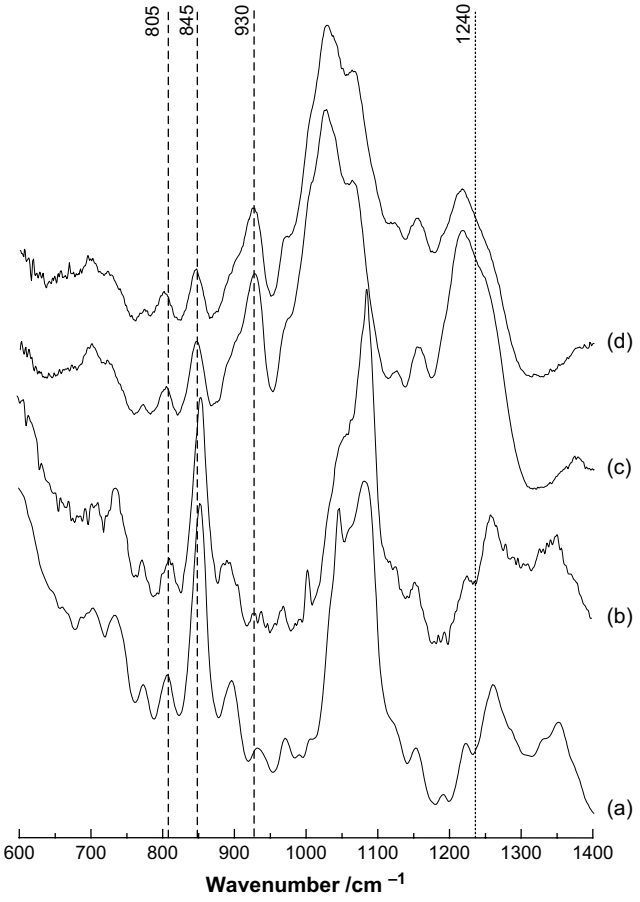

Fig. 4. Spectra of kappa/iota-carrageenan and of ground seaweed sample (*Chondracanthus teedei*, non-fructified thalli): FT-Raman (a and b, respectively) and FTIR-ATR (c and d, respectively).

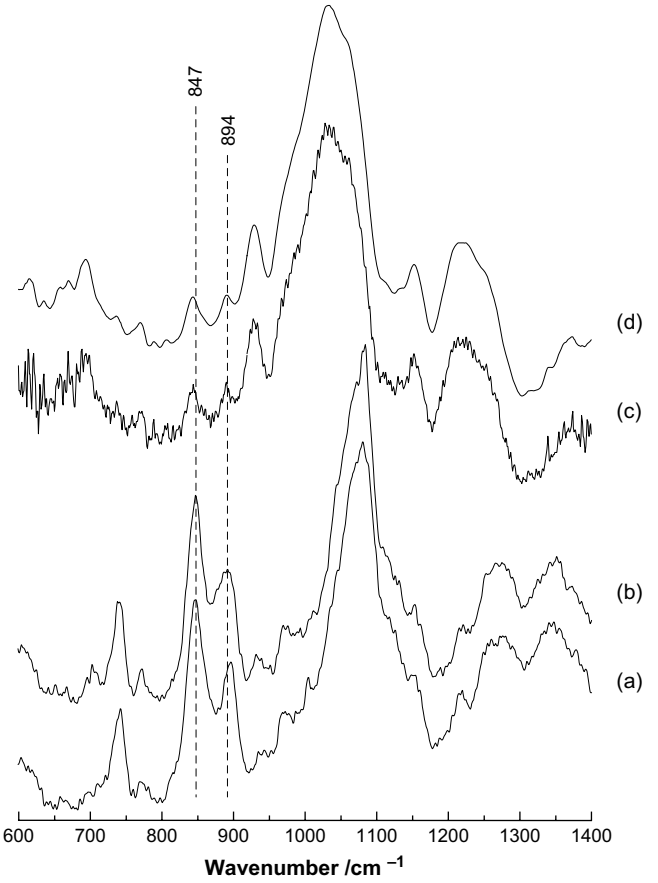

Fig. 5. Spectra of ground seaweed sample (*Betaphycus gelatinum*) and of kappa/beta-hybrid carrageenan alkali-extracted from *B. gelatinum*: FT-Raman (a and b, respectively) and FTIR-ATR (c and d, respectively).

The FTIR-ATR and FT-Raman spectra of lambda-carrageenan and ground *C. crispus* tetrasporophytes are shown in Fig. 6. These samples present high sulphate content as indicated by the broad band between 820 and 830 cm^{-1} in FTIR-ATR spectra. The *C. crispus* and lambda-carrageenan FT-Raman spectra show two combined peaks between 815 and 830 cm^{-1}.

Fig. 7 presents the FTIR-ATR and FT-Raman spectra of xi/theta-hybrid carrageenan of *C. teedei* var. *lusitanicus* tetrasporophyte. The FTIR-ATR spectra show a broad relatively intense band in the 800–850 cm^{-1} spectral region, with maximum at ca. 825 cm^{-1}. On the other hand, the FT-Raman spectra evidence two well-defined low-intensity bands in the same region, at 815 and 850 cm^{-1}. The occurrence of a band at 825 cm^{-1} has been related with the presence theta-carrageenan (Chopin et al., 1999), while the Raman bands at 815 and 850 cm^{-1} evidence the presence of xi-carrageenan.

On the whole, the presented results show that combination of FTIR and Raman spectroscopy allows the identification of the main

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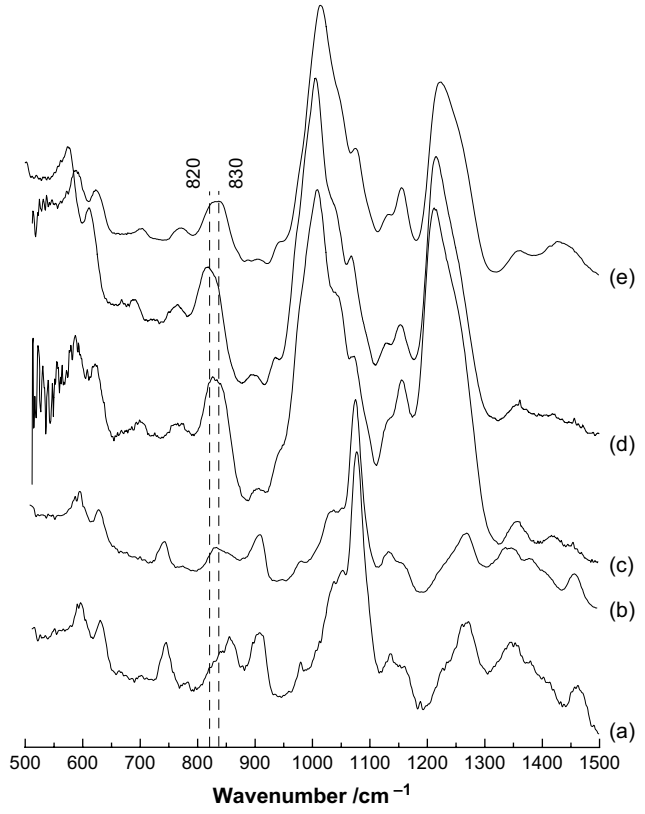

Fig. 6. FT-Raman (a) and FTIR-ATR (c) spectra of commercial lambda-carrageenan; FT-Raman (d) spectra of alkali-extracted carrageenan (*Chondrus crispus*, cultivated strain); FT-Raman (b) and FT-ATR (e) of ground seaweed sample (*C. crispus*, tetrasporophyte).

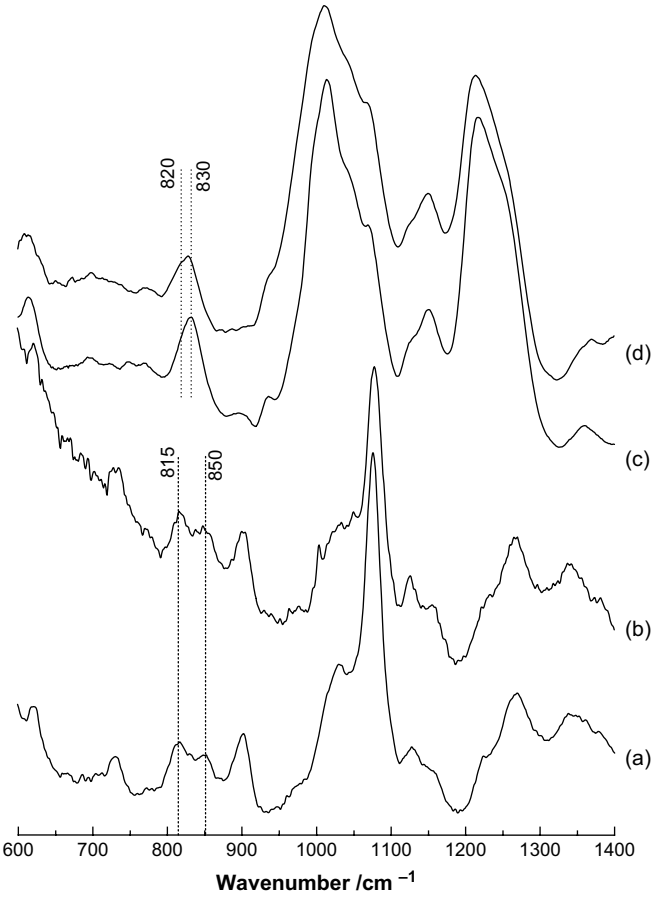

Fig. 7. Spectra of xi/theta-carrageenan and of ground seaweed sample (*Chondracanthus teedei* var. *lusitanicus*, tetrasporophyte): FT-Raman (a and b, respectively) and FTIR-ATR (c and d, respectively).

carrageenan composing the natural seaweeds. The principal spectral features (infrared absorptions and Raman bands) used for this discrimination are listed in Tables 1 and 2. The carrageenans are identified by the Greek lettering and by the letter code proposed by Knutsen, Myslabodski, Larsen, and Usov (1994).

All carrageenans considered but one (beta-carrageenan is the only exception) present a broad band in the 1240–1260 cm^{-1} spectral region ascribed to the S=O stretching vibration of the sulphated groups, being correlated to the sulphate content of the sample. Non-sulphated galactose gives rise to a characteristic band at 890–900 cm^{-1}.

Two bands around 1070 and 930 cm^{-1} are associated with the presence of C3–O–C6 bridge of the anhydrogalactose residue. These bands are absent in the spectra of the mu-, nu-, lambda- and xi-carrageenan. On the other hand, the presence of the sulphate groups gives rise to characteristic bands with the frequency being dependent on the position of the sulphate ring within the galactose and 3,6-anhydrogalactose units. For instance, when the sulphate group occupies the 2-position within the 3,6-anhydrogalactose ring (iota- and theta-carrageenan) two bands are expected at ca. 905 and 805 cm^{-1}. On the other hand, if the C-6 position of galactose is sulphated as in mu-, nu-, lambda- and xi-carrageenan then the characteristic bands are expected around 820 and 867 cm^{-1}. Finally sulphation at the C4- and C2-positions of galactose is expected to give rise to bands around 845 and 830 cm^{-1}, respectively.

4. Conclusions

The FTIR spectroscopy is useful in the comparative study of carrageenan types; however, it also shows that the complementary use of IR and Raman spectroscopy provides relevant additional information, allowing a better interpretation of the vibrational spectra and a more accurate identification of diverse colloids and variants. In fact, due to the different selection rules, bands of weak intensity or even those absent in the IR spectra may appear as sharp and intense bands in the Raman spectra. This is particularly evident, for instance, in the spectra of kappa/beta-hybrid carrageenan and xi/theta-hybrid carrageenan (Pereira & Mesquita, 2004; Pereira et al., 2003).

Since the vibrational spectrometers are now standard equipment in many laboratories, the techniques described in this work are useful for study of carrageenophyte populations, in substitution of the traditional tests of iridescence and resorcinol (Brown, Neish, & Harwood, 2004; Pereira, 2004). These techniques are also useful for the development and the implementation of strategies of sustainable seaweed harvest, the evaluation of the natural seaweed composition (e.g. carrageenophytes and alginophytes) with industrial potential and the evaluation and control of the quality of the different batches of algal material harvested and/or cultivated. The increments of the knowledge of the polysaccharide composition of the different species contribute also for the development of the phycocolloid chemotaxonomy. These spectroscopic techniques are also useful to analyse the composition of pharmaceutical, cosmetic excipients and food ingredients.

The main advantage of combining these two methods is that we increase the accuracy of the analysis while maintaining the use of small sample volumes. Moreover, this technique can be used on seaweeds and thus for different field of research.

Acknowledgements

The authors acknowledge financial support from the Portuguese Foundation for Science and Technology – *IMAR-CMA (Institute of*

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Marine Research), Unidade de Química-Física Molecular and Laboratório Associado CICECO.

References

Brown, M. T., Neish, A., & Harwood, D. (2004). Comparison of three techniques for identifying isomorphic phases of *Chondrus crispus* (Gigartinaceae). *Journal of Applied Phycology, 16*(6), 447–450.

Chopin, T., Kerin, B. F., & Mazerolle, R. (1999). Phycocolloid chemistry as taxonomic indicator of phylogeny in the Gigartinales, Rhodophyceae: a review and current developments using Fourier transform infrared diffuse reflectance spectroscopy. *Phycological Research, 47*, 167–188.

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. *Carbohydrate Research, 246*, 51–59.

Correa-Diaz, F., Aguilar-Rosas, R., & Aguilar-Rosas, L. E. (1990). Infrared-analysis of 11 carrageenophytes from Baja-California, Mexico. *Hydrobiologia, 204*, 609–614.

Dyrby, M., Petersen, R. V., Larsen, B., Rudolph, B., Norgaard, L., & Engelsen, S. B. (2004). Towards on-line monitoring of the composition of commercial carrageenan powders. *Carbohydrate Polymers, 57*, 337–348.

Knutsen, S. H., Myslabodski, D. E., Larsen, B., & Usov, A. I. (1994). A modified system of nomenclature for red algal galactans. *Botanica Marina, 37*(2), 163–169.

Matsuhiro, B. (1996). Vibrational spectroscopy of seaweed galactans. *Hydrobiologia, 327*, 481–489.

Pereira, L. (2004). Estudos em macroalgas carragenófitas (Gigartinales, Rhodophyceae) da costa portuguesa – aspectos ecológicos, bioquímicos e citológicos. *Ph.D. Thesis. Departamento de Botânica – FCTUC* (p. 293). Coimbra: Universidade de Coimbra.

Pereira, L. (2006). Identification of phycocolloids by vibrational spectroscopy. In A. T. Critchley, M. Ohno, & D. B. Largo (Eds.), *World seaweed resources – an authoritative reference system*. ETI Information Services Ltd.

Pereira, L., & Mesquita, J. F. (2004). Population studies and carrageenan properties of *Chondracanthus teedei* var. *lusitanicus* (Gigartinaceae, Rhodophyta). *Journal of Applied Phycology, 16*(5), 369–383.

Pereira, L., Sousa, A., Coelho, H., Amado, A. M., & Ribeiro-Claro, P. J. A. (2003). Use of FTIR, FT-Raman and ^{13}C-NMR spectroscopy for identification of some seaweed phycocolloids. *Biomolecular Engineering, 20*(4–6), 223–228.

Pereira, L., van de Velde, F., & Mesquita, J. F. (2007). Cytochemical studies on underutilized carrageenophytes (Gigartinales, Rhodophyta). *International Journal of Biological and Biomedical Engineering, 1*(1), 1–5.

Rudolph, B. (2000). Seaweed products: red algae of economic significance. In R. E. Martin (Ed.), *Marine & freshwater products handbook* (pp. 515–529). Lancaster, PA: Technomic Pub. Co.

van de Velde, F., Pereira, L., & Rollema, H. S. (2004). The revised NMR chemical shift data of carrageenans. *Carbohydrate Research, 339*(13), 2309–2313.

van de Velde, F., & de Ruiter, G. A. (2002). Carrageenan. In E. J. Vandamme, S. D. Baets, & A. Steinbèuchel (Eds.), *Biopolymers v. 6. Polysaccharides II, polysaccharides from eukaryotes* (pp. 245–274). Weinheim; Chichester: Wiley-VCH.

Zinoun, M., & Cosson, J. (1996). Seasonal variation in growth and carrageenan content of *Calliblepharis jubata* (Rhodophyceae, Gigartinales) from the Normandy coast, France. *Journal of Applied Phycology, 8*(1), 29–34.

Please cite this article in press as: Pereira, L., et al., Identification of selected seaweed polysaccharides (phycocolloids) by vibrational..., Food Hydrocolloids (2009), doi:10.1016/j.foodhyd.2008.11.014