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Research Article

Analysis by Vibrational Spectroscopy of Seaweed Polysaccharides with Potential Use in Food, Pharmaceutical, and Cosmetic Industries

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Polysaccharides present in several seaweeds (*Kappaphycus alvarezii*, *Calliblepharis jubata*, and *Chondrus crispus*—Gigartinales, Rhodophyta; *Gelidium corneum* and *Pterocladiella capillacea*—Gelidiales, Rhodophyta; *Laurencia obtusa*—Ceramiales, Rhodophyta; *Himanthalia elongata*, *Undaria pinnatifida*, *Saccorhiza polyschides*, *Sargassum vulgare*, and *Padina pavonica*—Phaeophyceae, Ochrophyta) are analyzed by spectroscopic techniques. The nature of the polysaccharides (with extraction and without any type of extraction) present in these seaweeds was determined with FTIR-ATR and FT-Raman analysis of extracted phycocolloids and ground dry seaweed.

1. Introduction

Many species of seaweed (marine macroalgae) are used as food and they have also found use in traditional medicine because of their perceived health benefits. Seaweeds are rich sources of sulphated polysaccharides, including some that have become valuable additives in the food industry because of their rheological properties as gelling and thickening agents (e.g., alginates, agar, and carrageenan). Sulphated polysaccharides are recognized to possess a number of biological activities including anticoagulant, antiviral, anti-tumor, anti-inflammatory, and immunostimulating activities that might find relevance in nutraceutical/functional food, cosmetic, and pharmaceutical applications [1].

Some 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 (Heterokontophyta, Phaeophyceae) produce uronates (alginates) and other sulphated polysaccharides (e.g., fucoidan and laminaran) [2–8].

The different phycocolloids used in food industry as natural additives are (European codes of phycocolloids)

(i) alginic acid—E400,

(ii) sodium alginate—E401,

(iii) potassium alginate—E402,

(iv) ammonium alginate—E403,

(v) calcium alginate—E404,

(vi) propylene glycol alginate—E405,

(vii) agar—E406,

(viii) carrageenan—E407,

(ix) semirefined carrageenan or "processed *Eucheuma* seaweed"—E407A.

Carrageenan and agar (Figure 1) are the principal sulphated polysaccharides produced by red seaweeds (Rhodophyta);

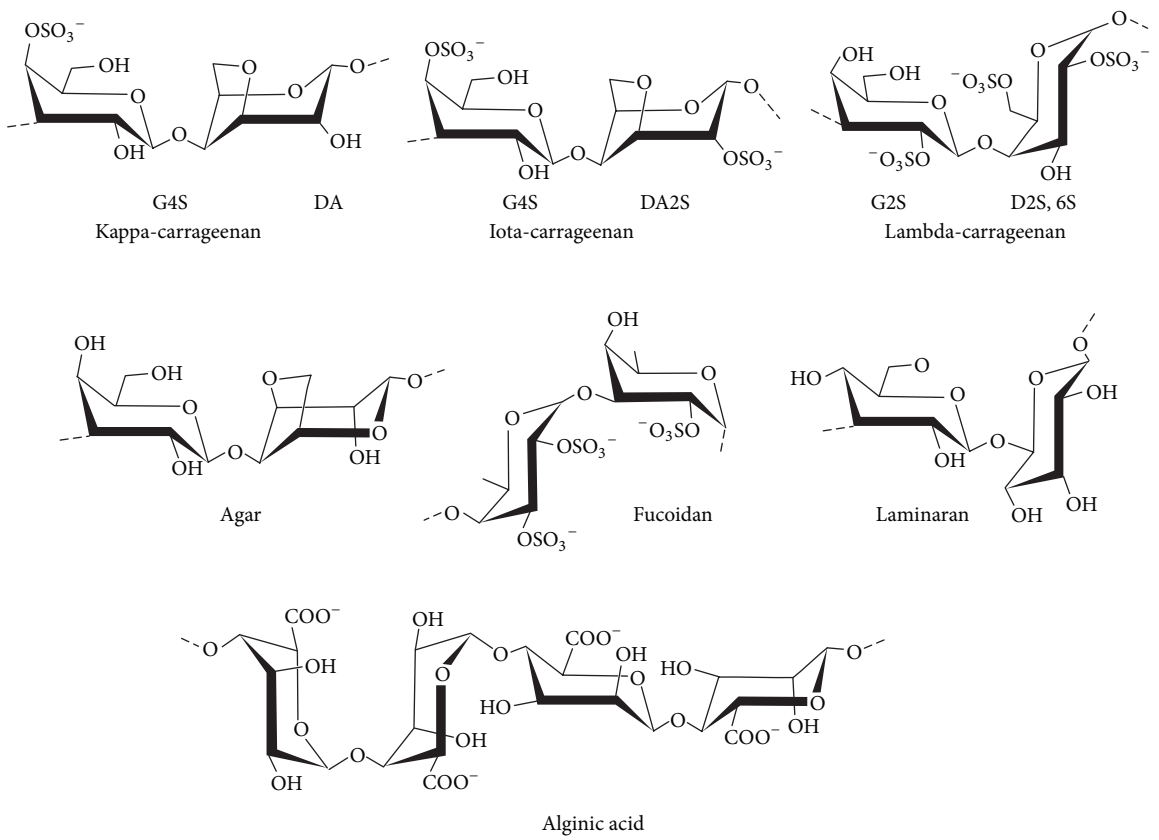

Figure 1: Idealized structures of the chemical units of kappa-, iota-, and lambda-carrageenan, agar, alginic acid (M = mannuronic acid and G = guluronic acid), fucoidan and laminaran [13, 14].

the main difference between the highly sulphated carrageenans from the less sulphated agars is the presence of D-galactose and anhydro-D-galactose in carrageenans and of D-galactose, L-galactose, or anhydro-L-galactose in agars.

The structure of the various types of carrageenans is defined by the number and position of sulphate groups, the presence of 3,6-anhydro-D-galactose, and conformation of the pyranose ring. There are about fifteen idealized carrageenan structures traditionally identified by Greek letters [9].

The commercial carrageenans are normally divided into three main types: kappa-, iota-, and lambda-carrageenans. Generally, seaweeds do not produce these idealized and pure carrageenans but more likely a range of hybrid structures. 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 [10].

Different types of carrageenan are obtained from different species of the Gigartinales (Rhodophyta). Kappa-carrageenan is predominantly obtained by extraction from the cultivated tropical seaweed *Kappaphycus alvarezii* (known in the trade as "cottonii"). *Eucheuma denticulatum* (trade name "spinosum") is the main species for the production of iota-carrageenan. Lambda-carrageenan is obtained from different species from the genera *Gigartina* and *Chondrus* (trade name "Irish moss") [11].

The rheological properties of the gelling carrageenans (e.g., kappa and iota) are quite distinct: the kappa type forms gels that are hard, strong, and brittle, whereas iota-carrageenan forms soft and weak gels. The common feature of these carrageenans is the anhydrogalactose bridge of the 4-linked galactose residue, DA and DA2S, respectively, which adopts the $^1C_4$-chair conformation. This conformation is crucial for the formation of the helical structure and, thereby, for the ability to form a gel. Lambda-carrageenan and the precursors mu- and nu-carrageenan lack the 3,6-anhydrobridge and, therefore, the 4-linked residue adopts the $^4C_1$-chair conformation, which disturbs the helical conformation. Thus, lambda-carrageenan acts simply as a thickening agent [11].

Agar (Figure 1) was the first colloid to be developed and it has applications as a gelling agent for food and also as an inert support medium for microbial culture. This polysaccharide is the dried hydrophilic, colloidal substance extracted commercially from certain marine algae of the phylum Rhodophyta. The most important commercial agarophyte genera are *Gelidium*, *Pterocladiella*, *Gelidiella,* and *Gracilaria*. Agar has also been found in species of *Ceramium*, *Phyllophora*, *Ahnfeltia*, *Campylaephora*, *Acanthopholis,* and *Gracilariopsis*. It is a polysaccharide, consisting primarily of D- and L-galactose units. About every tenth D-galactopyranose unit contains a sulphate ester group. Calcium, magnesium, potassium, or sodium cations are also

associated with the polysaccharide. Agar may be separated into two fractions. One is a neutral polymer, agarose, composed of repeating units, referred to as agarobiose, and of alternating 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose. The second fraction has agaropectin, a more complicated structure. It contains residues of sulphuric, pyruvic, and uronic acids, in addition to D-galactose and 3,6-anhydro-L-galactose [10, 12].

Alginic acid (Figure 1) was discovered in 1883 by E. C. C. Stanford, a British pharmacist who called it algin. In seaweeds, algin is extracted as a mixed salt of sodium and/or potassium, calcium, and magnesium. The exact composition varies with algal species. Since Stanford discovered algin, the name has been applied to a number of substances, such as alginic acid and all alginates, derived from alginic acid. 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 [13–15]. This polysaccharide is derived from several genera of brown algae (e.g., mixed Fucales and Laminariales) that are utilized as raw materials by commercial alginate producers; these include *Macrocystis*, *Laminaria*, *Lessonia*, *Ascophyllum*, *Alaria*, *Ecklonia*, *Eisenia*, *Nereocystis*, *Sargassum*, *Cystoseira* and *Fucus*, with *Macrocystis pyrifera* and *Ascophyllum nodosum* being the principal sources of the world's alginate supply. The intercellular mucilage in these seaweeds has been regarded as the principal site of algin, although it has also been found to occur in the cell walls. Alginic acid is a complex organic compound composed of D-mannuronic acid and L-guluronic acid monomers [15–17].

Fucans (Figure 1) are sulphated polysaccharides that are composed of a fucose backbone. One of the best studied fucans from brown algae is fucoidan, which was first isolated by Kylin in 1913 [18]. The fucoidan from *Fucus vesiculosus* has been available commercially for decades (Sigma-Aldrich Chemical Company, St. Louis, MO, USA). Early work on its structure showed that it contained primarily $(1 \rightarrow 2)$ linked 4-*O*-sulphated fucopyranose residues. However, 3-linked fucose with 4-sulphated groups were subsequently reported to be present on some of the fucose residues. Additionally, it was determined to contain branches every 2-3 fucose residues. Subsequently, Chevolot and colleagues reported that the fucoidan from *F. vesiculosus* and *Ascophyllum nodosum* contains a predominant disaccharide motif containing sulphate at the 2-position of the 3-linked fucose and sulphate groups on the 2- and 3-positions of the 4-linked fucose [19].

Laminaran (Figure 1) is a small glucan present in either soluble or insoluble forms. The first form is characterized by complete solubility in cold water, while the other is only soluble in hot water [20, 21]. This polysaccharide is composed of D-glucose with b-(1,3) linkages, with b-(1,6) intrachain branching [6–8].

In this work, a combined FTIR-ATR and FT-Raman spectroscopy analysis was used to identify the main seaweed polysaccharides, namely, alginate, fucoidan, laminaran, agar, and kappa-, iota-, and lambda-carrageenans. Therefore, vibrational spectroscopy (FTIR-ATR and FT-Raman) is proposed as a useful tool for the cosmetic, pharmaceutical, and food industries to check the phycocolloid quality of a raw seaweed material by a quick and nondestructive method [5, 16].

2. Materials and Methods

2.1. Algal Material and Standard Samples of Hydrocolloids. Specimens of red algae (Rhodophyta) *Kappaphycus alvarezii* (Gigartinales) were collected in the Philippines. Specimens of red algae (Rhodophyta) *Calliblepharis jubata, Chondrus crispus* (Gigartinales), and *Gelidium corneum* (Gelidiales) and the brown algae (Phaeophyceae) *Saccorhiza polyschides* (Tilopteridales) were collected in the central zone of the western coast of Portugal. *Pterocladiella capillacea* (Gelidiales, Rhodophyta), *Laurencia obtusa* (Ceramiales, Rhodophyta), *Padina pavonica* (Dictyotales, Phaeophyceae), and *Sargassum vulgare* (Fucales, Phaeophyceae) were collected in the Mediterranean (Egypt). The edible brown seaweeds (Phaeophyceae) *Himanthalia elongata* (Fucales), and *Undaria pinnatifida* (Laminariales) were obtained from Algamar (Galicia, Spain).

Standard samples were obtained from Sigma (kappa-carrageenan, type III, C-1263; iota-carrageenan, type V, C-4014; alginic acid, A0682), TAAB Laboratories (agar, A010), and CP Kelco (pure lambda-carrageenan).

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) [5, 16, 22].

2.3. FTIR-ATR and FT-Raman Analysis. 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}.

3. Results and Discussion

The assignments of the IR spectra were mostly based on previous works [4, 5, 9, 16, 23]. The Raman spectra were assigned based on the IR information and on the comparison between samples of known composition.

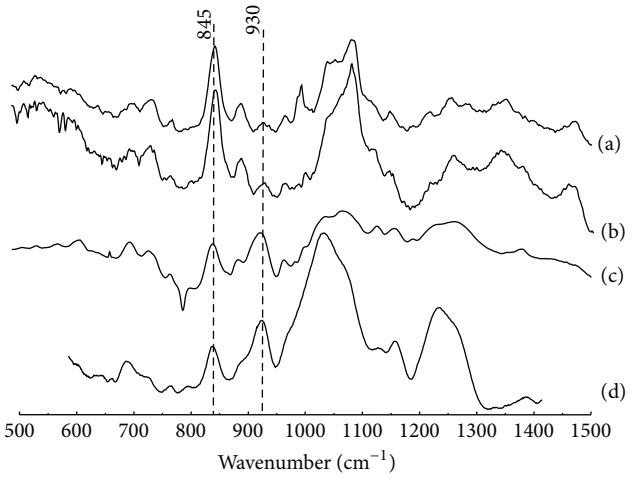

Figure 2: Spectra of commercial kappa-carrageenan and of ground seaweed sample (*Kappaphycus alvarezii*): FT-Raman ((a) and (b), resp.) and FTIR ((c) and (d), resp.).

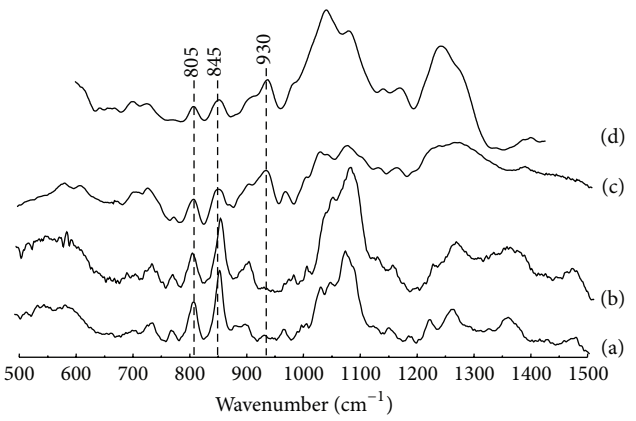

Figure 3: Spectra of commercial iota-carrageenan and of ground seaweed sample (*Calliblepharis jubata*): FT-Raman ((a) and (b), resp.) and FTIR ((c) and (d), resp.).

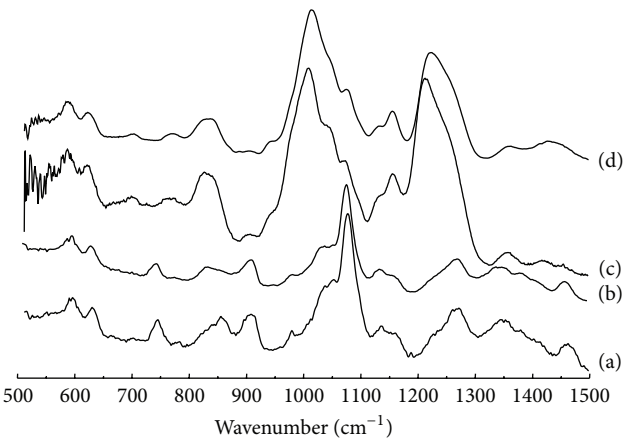

Figure 4: Spectra of commercial lambda-carrageenan and of ground seaweed sample (*Chondrus crispus* tetrasporophyte): FT-Raman ((a) and (b), resp.) and FTIR ((c) and (d), resp.).

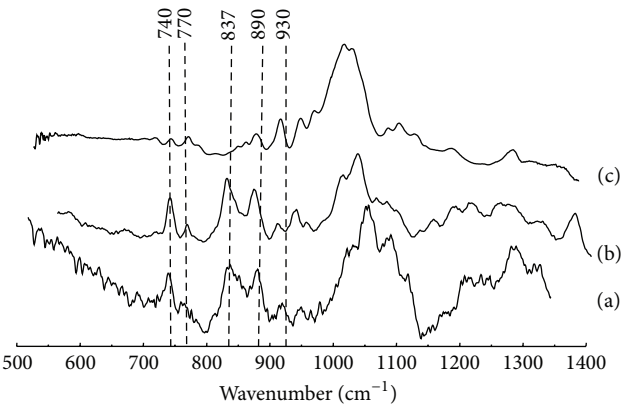

Figure 5: FT-Raman spectra of ground seaweed *Gelidium corneum* (a) and commercial agar (b) andFTIR-ATR spectra of ground seaweed *Gelidium corneum* (c).

3.1. Identification of Carrageenan. The FTIR-ATR and FT-Raman spectra of *Kappaphycus alvarezii* were compared with those of commercial kappa-carrageenan in Figure 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) [5, 16].

Figure 3 presents the FTIR-ATR and FT-Raman spectra of iota-carrageenan and of *Calliblepharis jubata*. 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 [5, 16].

The FTIR-ATR and FT-Raman spectra of lambda-carrageenan and ground *Chondrus crispus* tetrasporophytes are shown in Figure 4. 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 the two combined weak bands between 815 and 830 cm^{-1} [5, 16].

3.2. Identification of Agar. Agars differ from carrageenans as they have the L-configuration for the 4-linked galactose residue; nevertheless, they have some structural similarities with carrageenans. The characteristic broad band of sulphate esters in general [9] between 1210 to 1260 cm^{-1} (Figures 2, 3, and 4) was much stronger in carrageenan than in agar (Figures 5 and 6). Especially in the anomeric region (700–950 cm^{-1}) agar and carrageenan showed several similar bands. Thus, the strong IR band at 930 cm^{-1} (Figures 5(c) and

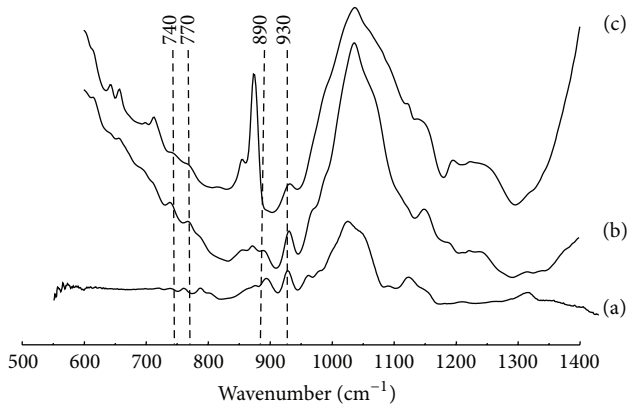

Figure 6: FTIR-ATR spectra of commercial agar (a), ground seaweed *Pterocladiella capillacea* (b), and *Laurencia obtusa* (c).

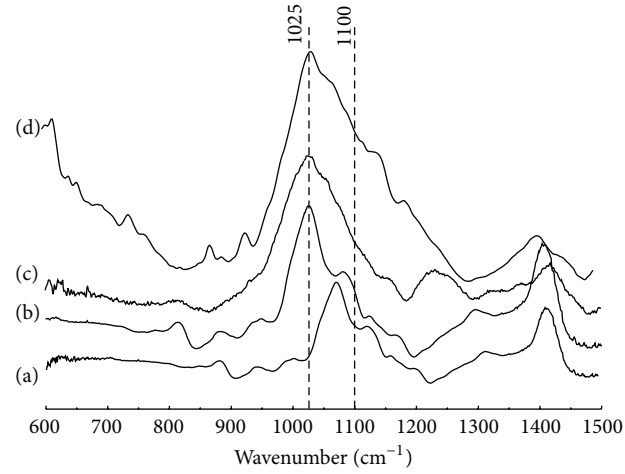

Figure 7: FT-Raman spectra of commercial alginate (a) and FTIR spectra of commercial alginate (b) and *Saccorhiza polyschides* (c). FTIR-ATR spectra of ground seaweed *Himanthalia elongata* (d).

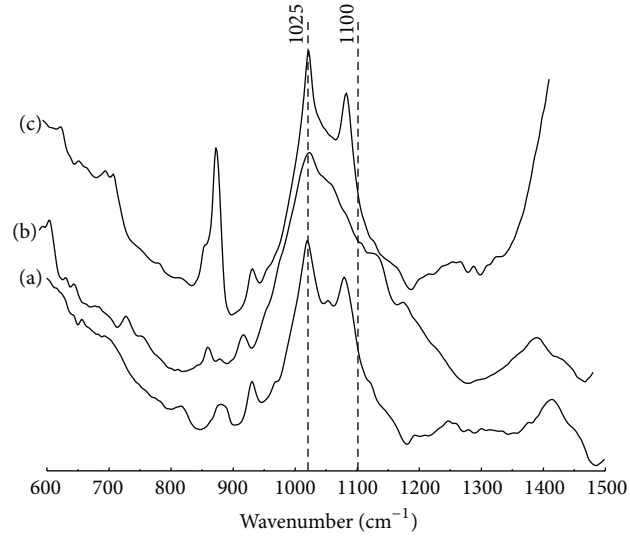

Figure 8: FTIR-ATR spectra of ground seaweed *Undaria pinnatifida* (a), *Padina pavonica* (b), and *Sargassum vulgare* (c).

6(a)–6(c)) assigned to the presence of 3,6-anhydrogalactose was common to agar and carrageenans; the band at 890 cm^{-1} (Figures 5(a), 5(b), and 6) corresponded to anomeric CH of β-galactopyranosyl residues and Raman bands at 770, 740 (strong in the FT-Raman spectra and weak in the FTIR-ATR) are assigned to the skeleton bending of pyranose ring [23–25] both in agar and carrageenans. Also, the bands at 1010–1030 cm^{-1} (Figures 5(c) and 6(d)–6(f)) may be assigned to C–O and C–C stretching vibrations of pyranose ring common to all polysaccharides. So, the main polysaccharide composition of *Gelidium corneum* and of *Pterocladiella capillacea* is agar [23, 26, 27].

The FT-Raman spectra (Figures 5(a) and 5(b)) show a strong band centred at 837 cm^{-1}, which is absent in the FTIR spectra. After Matsuhiro [24], this band is associated with the CH vibration coupled with C–OH related modes of α residues. Moreover, the spectral feature at 890 cm^{-1}, also particularly intense in the FT-Raman spectra, is mainly associated with vibrational modes of the β-galactose residues.

Laurencia obtusa (Figure 6(f)) presents a complex agar-like sulphated galactan. These polysaccharides belong to the agar group, being agarose derivatives with a rather high content sulfate groups and with a reduced amount of 3,6-anhydro-L-galactose residues (700–950 cm^{-1}) [28].

3.3. Polysaccharides from Brown Algae. The main polysaccharide found in studied brown seaweeds (Phaeophyceae) was alginate, a linear copolymer of mannuronic (M) and guluronic acid (G). Different types of alginic acid present different proportions and/or different alternating patterns of guluronic (G) and mannuronic (M) units. The presence of these acids can be identified from their characteristic bands in the vibrational spectra; in accordance with Mackie [25] these phycocolloids show two characteristic bands in IR spectra: 808 cm^{-1}, assigned to M units, and 787 cm^{-1}, assigned to G units. However, Matsuhiro and coworkers, in work with specimens of *Lessonia* genus, assign both bands to G units [29, 30]. Filipov and Kohn [31] propose that M/G ratios of the different samples can be estimated from the ratio of absorbance of the bands at 1320 and 1290 cm^{-1} in FTIR spectra. According to Sakugawa and collaborators [32] the M/G concentration ratio characterizing a certain alginate sample can be inferred from the relative intensity ratio of the two bands 1030/1080 cm^{-1}, in calcium alginate and 1019/1025 cm^{-1}, in manganese alginate. In accordance with the same authors, the absorbance at 1030 cm^{-1} directly reflects the change of mannuronate concentration of calcium alginate and the 1025 cm^{-1} is attributed to the OH bending of guluronate [32].

Alginate M/G ratio was tentatively estimated from the 1030/1080 cm^{-1} band ratio in infrared spectra, suggesting higher values of mannuronic than guluronic acid blocks (M/G > 1) in *Himanthalia elongata* (Figure 7(d)). However, the FTIR spectra of *Saccorhiza polyschides* (Figure 7(c)) show

an intense broad band centred at 1025 cm^{-1}, indicating that the samples considered are particularly rich in guluronic acid. According to our spectrum on *U. pinnatifida* (old adult thallus, Figure 8(a)), the relative amounts of both mannuronate and guluronate residues were similar, and result in accordance with other published works [33].

Our spectra suggesting higher values of guluronic than mannuronic acid blocks in *Padina pavonica* (Figure 8(b)); *Sargassum vulgare* spectra (Figure 8(c)) suggesting the presence of similar amounts of the mannuronate and guluronate residues, results in accordance with other published works [33–35].

Some brown algae (see Figures 7(c) and 8(a), *Saccorhiza polyschides* and *Undaria pinnatifida,* resp.) also exhibited a broad band around 1220–1260 cm^{-1}, assigned to the presence of sulphate ester groups (S=O) which is a characteristic component in fucoidan and sulphated polysaccharides other than alginate in brown seaweeds. *Padina pavonica* and *Sargassum vulgare* (see Figures 8(b) and 8(c)) also exhibited a broad band (around 1195–1237 cm^{-1} for *Padina* and 1210–1280 cm^{-1} for *Sargassum*) assigned to (S=O) which is characteristic component in fucoidan (main water soluble sulphated polysaccharide); however, *Sargassum vulgare* contains more amounts of fucoidan than in *Padina pavonica*, whereas, both seaweed species contain little amounts of laminaran [2, 6–8, 20, 21, 36].

4. Conclusion

Vibrational FTIR-ATR spectroscopy (FTIR-ATR and FT-Raman) is a useful tool in the food, pharmaceutical, and cosmetics industries for a preliminary identification of the main sulphated polysaccharides (namely, alginate, laminaran, fucoidan, agar, and carrageenan) produced by edible brown and red seaweeds, by a rapid and non-destructive method.

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