CHAPTER 7

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

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1 Introduction

1.1 Vibrational Spectroscopy

In order to determine the chemical nature of the compounds present in seaweed, vibrational spectroscopy arises as a useful tool, as it can reveal detailed information concerning the properties and structure of materials at a molecular level. Until now, this type of analysis required the extraction of polysaccharides and other compounds, through lengthy and complicated procedures. With the development of FTIR diffuse-reflectance spectroscopy (DRIFTS) it became possible to directly analyze ground, dried seaweed material (Chopin and Whalen 1993). On the other hand, the development of Raman Spectroscopy with affordable low energy lasers (FT-Raman) allowed the use of this complementary technique in the study of the same ground samples.

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Pereira and collaborators (2003, 2006, 2009, 2013) developed an analysis technique based on FTIR-ATR (attenuated total reflectance) and FT-Raman spectroscopy, which allowed for the accurate identification of diverse polysaccharides (namely the phycocolloids) and other natural compounds present in seaweeds.

1.1.1 Infrared spectroscopy

Infrared spectroscopy was until recently the most widely used vibrational technique for studying natural products. In the Fourier transform instruments, all frequencies are scanned simultaneously, making data collection extremely rapid, allowing to collect several scans in a short time, and resulting in an improved signal-to-noise ratio of final spectrum. In the spectrometer, an infrared source emits radiation with a range of frequencies that is then passed through the sample. Particular chemical bands in the sample absorb infrared radiation of specific frequencies from the beam (Fig. 1), and this is plotted as an absorbance spectrum against wave number.

This technique presents several advantages, the most important one being its sensitivity: the infrared absorption is efficient and a good infrared spectrum can be obtained from a small amount of sample.

Infrared absorption intensity depends on the change of dipole moment during the molecular vibration. In this way, the stronger IR signals are observed for vibrations involving polar functional groups (with permanent dipole moment), such as OH group (e.g., in water and carbohydrates)

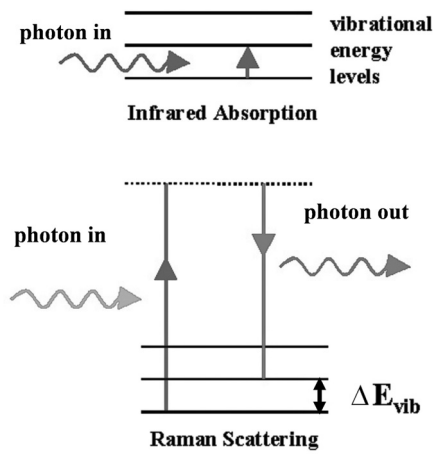

Figure 1. Diagram that illustrates infrared absorption and Raman scattering (Adapted from Pereira 2006).

Color image of this figure appears in the color plate section at the end of the book.

and C=O group (e.g., in aldehydes, ketones, and amides). This makes this technique quite suitable for studying these constituents and their intermolecular interactions (e.g., hydrogen-bonding) in organic and biological systems.

Usually, IR spectroscopy requires some sample preparation for transmission (e.g., dilution of sample in KBr pellets), but allows the use of pure samples, depending on the accessories used (ATR, IRRAS, DRIFTS).

1.1.2 Raman spectroscopy

In contrast with FTIR, the application of traditional Raman spectroscopy (Fig. 1) was limited until recently, due to the laser-induced fluorescence (strong background signal which is detected when some samples, such as biochemical compounds, are excited with visible lasers) and risk of sample destruction by the high energy of visible radiation. The use of Nd:YAG lasers operating at 1064 nm (infrared, lower energy radiation) has been generalized to decrease the fluorescence level and sample damage. Opto-electronic devices have progressed dramatically in the past decade as a consequence of major achievements in solid-state technology. As a result compact, efficient, and reliable diode lasers are now available from the visible to the infrared that have been demonstrated to work properly in Raman instruments in combination with suitable filter sets (Pereira 2006).

Raman spectroscopy comprises the family of spectral measurements made on molecular media based on inelastic scattering of monochromatic radiation. In a simplified description, part of the energy of the incident photon is left on the molecule, so that the scattered photon is of lower energy than the incident photon (Fig. 1). The energy difference between the incident and scattered photons is the exact measure of the energy difference between molecular vibrational levels—the same levels that are directly probed by infrared spectroscopy.

In Raman spectroscopy, the intensity of the radiation-matter interaction depends on the molecular polarizability change during the vibrational motion—which differs from the change of dipole moment that determines IR absorption. In this way, these two techniques provide complementary information: vibrational modes that are weak or not observed in one technique can usually be observed in the other.

1.2 Seaweed Polysaccharides

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) (see Table 1). Sulphated polysaccharides are recognized to possess a number of biological activities

Table 1. Applications of Macroalgae Phycocolloids (adapted from van de Velde and de Ruiter 2002, Dhargalkar and Pereira 2005, Pereira 2004, 2008).

Use	Phycocolloid	Function
Food additives		
Baked food	Agar Kappa, Iota, Lambda	Improving quality and controlling moisture
Beer and wine	Alginate Kappa	Promotes flocculation and sedimentation of suspended solids
Canned and processed meat	Alginate Kappa	Hold the liquid inside the meat and texturing
Cheese	Kappa	Texturing
Chocolate milk	Kappa, lambda	Keep the cocoa in suspension
Cold preparation puddings	Kappa, Iota, Lambda	Thicken and gelling
Condensed milk	Iota, lambda	Emulsify
Dairy Creams	Kappa, iota	Stabilize the emulsion
Fillings for pies and cakes	Kappa	Give body and texture
Frozen fish	Alginate	Adhesion and moisture retention
Gelled water-based desserts	Kappa + Iota Kappa + Iota + CF	Gelling
Gums and sweets	Agar Iota	Gelling, texturing
Hot preparation flans	Kappa, Kappa + Iota	Gelling and improving taste
Jelly tarts	Kappa	Gelling
Juices	Agar Kappa, Lambda	Viscosity, emulsifier
Low calorie gelatins	Kappa + Iota	Gelling
Milk ice-cream	Kappa + GG, CF, X	Stabilize the emulsion and prevent ice crystals formation
Milkshakes	Lambda	Stabilize the emulsion
Salad dressings	Iota	Stabilize the suspension
Sauces and condiments	Agar Kappa	Thicken
Soymilk	Kappa + iota	Stabilize the emulsion and improve the taste
Cosmetics		
Shampoos	Alginate	Vitalization interface
Toothpaste	Carrageenan	Increase viscosity

Table 1. contd....

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Use	Phycocolloid	Function
Lotions	Alginate	Emulsification, elasticity and skin firmness
Lipstick	Alginate	Elasticity, viscosity
Medicinal and Pharmaceutical uses		
Dental mould	Alginate	Form retention
Laxatives	Alginate Carrageenan	Indigestibility and lubrication
Tablets	Alginate Carrageenan	Encapsulation
Metal poisoning	Carrageenan	Binds metal
HSV	Alginate	Inhibit virus
Industrial and Lab Uses		
Paints	Alginate	Viscosity and suspension, glazing
Textiles	Agar, Carrageenan	Sizing and glazing
Paper making	Alginate, Agar, Carrageenan	Viscosity and thickening
Analytical separation	Alginate, Carrageenan	Gelling
Bacteriological media	Agar	Gelling
Electrophoresis gel	Agar, Carrageenan	Gelling

Non-seaweed colloids: CF—Carob flour; GG—Guar gum; X—Xanthan

including anticoagulant, antiviral, and immune-inflammatory activities that might find relevance in nutraceutical/functional food, cosmetic and pharmaceutical applications (Pereira 2011, Table 2).

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) (Peat et al. 1958, Rinaudo 2002, Costa et al. 2010, Rioux et al. 2010, Ale et al. 2011, Jiao et al. 2011).

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

- Alginic acid–E400
- Sodium alginate–E401
- Potassium alginate–E402
- Ammonium alginate–E403
- Calcium alginate–E404
- Propylene glycol alginate–E405

Table 2. Summary of bioactive activity of some seaweed compounds (polysaccharides and phenolic compounds).

Category	Compounds	Seaweed source	Potential health benefit	Reference
Polyphenols	Flavonoids	*Palmaria palmata*	At high experimental concentrations that would not exist *in vivo*, the antioxidant abilities of flavonoids *in vitro* are stronger than those of vitamin C and E	Bagchi et al. 1999, Yuan et al. 2005
	Phlorotannins	Brown algae	Antioxidant activity of polyphenols extracted from brown and red seaweeds has already been demonstrated by *in vitro* assays; anti-inflammatory effect	Nakamura et al. 1996, Shin et al. 2006, Shibata et al. 2008, Wijesekara et al. 2010, Ngo et al. 2011
			Algicidal and bactericidal effect	Nagayama et al. 2002, 2003, Ngo et al. 2011
Polysaccharides and dietary fibers	Agars, carrageenans, ulvans and fucoidans	Red and brown algae	These polysaccharides are not digested by humans and therefore can be regarded as dietary fibers	Lahaye and Thibault 1990, Lahaye 1991, Costa et al. 2010
	Ulvan	*Ulva pertusa*	Antihyperlipidemic effects	Fujiwara-Arasaki et al. 1984, Pengzhan et al. 2003
	Carrageenan, fucoidan	Red algae (carrageenophytes), *Undaria pinnatifida*, brown algae	Antitumor and anti-viral	Haijin et al. 2003, Pengzhan et al. 2003, Choosawad et al. 2005, Nisizawa 2006, Hemmingson et al. 2006,Yuan and Song 2005, Yuan et al. 2010, Ngo et al. 2011
	Carrageenan (lambda, iota and nu variants)	Red algae (carrageenophytes)	Anti-viral, anti-HSV and anti-HIV	Spieler 2002, Smit 2004, Jiao et al. 2011

Table 2. contd....

Table 2. contd.

Category	Compounds	Seaweed source	Potential health benefit	Reference
	Fucoidan	Brown algae	Anticoagulant and antithrombotic activity	Smit 2004, Li et al. 2008, Ngo et al. 2011
			Antitumor and immunomodulatory activity	Choosawad et al. 2005, Li et al. 2008, Kim et al. 2010
			Antiviral and anti-HIV	Sugawara et al. 1989, Béress et al. 1993, Witvrouw and de Clercq 1997, Feldman et al. 1999, Smit 2004, Li et al. 2008, Cornish and Garbary 2010
	Fucoidan	*Fucus vesiculosus* *Saccharina japonica*	Hypolipidemic effect	Vázquez-Freire et al. 1996, Huang et al. 2010

Abbreviations: HIV—Human immunodeficiency virus; HSV—Herpes simplex virus

- Agar–E406
- Carrageenan–E407
- Semi-refined carrageenan or "processed *Eucheuma* seaweed"–E407A

1.2.1 Ulvan

Ulvan represents 8–29% of the algae dry weight and is produced by species belonging to the phylum Chlorophyta (green algae), mostly belonging to the class Ulvophyceae (Robic et al. 2009). It is mainly made up of disaccharide repeating sequences composed of sulfated rhamnose and glucuronic acid, iduronic acid, or xylose (Percival and McDowell 1967, Quemener et al. 1997). The two major repeating disaccharides are aldobiuronic acids designated as: type A, ulvanobiuronic acid 3-sulfate (A3s) and type B, ulvanobiuronic acid 3-sulfate (B3s) (Fig. 2). Partially sulfated xylose residues at O-2 can also occur in place of uronic acids (Fig. 2). Low proportions of galactose, glucose, mannose, and protein are also generally found in ulvan. Aditionally, minor repeat units have been reported that contain sulfated xylose replacing the iduronic acid or glucuronic acid (Lahaye and Robic 2007, Jiao et al. 2011).

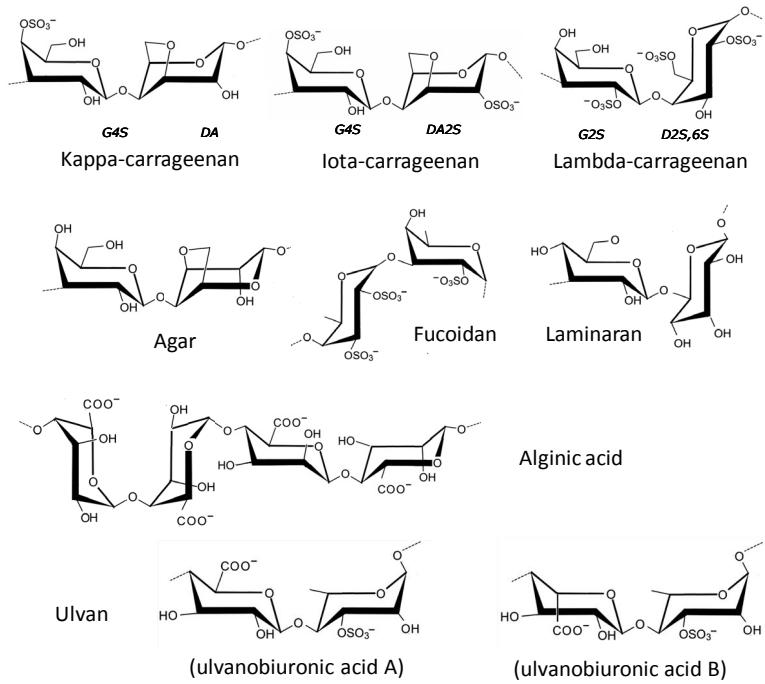

Figure 2. Idealized structures of the chemical units of kappa-, iota-, and lambda-carrageenan, agar, alginic acid, fucoidan, laminaran and ulvan.

1.2.2 Laminaran

Laminaran (Fig. 2) is a small glucan present in either soluble or insoluble form. The first form is characterized by complete solubility in cold water, while the other is only soluble in hot water (Kylin 1913, Chevolot et al. 2001). This polysaccharide is composed of D-glucose with β-(1,3) linkages, with β-(1,6) intra-chain branching (Barry 1939, Peat et al. 1958, Rioux et al. 2010).

1.2.3 Fucans

Fucans (Fig. 2) 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 (Kylin 1913). The fucoidan from *Fucus vesiculosus* has been available commercially for decades (Sigma-Aldrich Chemical Company, St. Louis). Early work on its structure showed that it contained primarily (1→2) linked 4-*O*-sulphated fucopyranose residues. However, 3-linked fucose with 4-sulphated groups was subsequently reported to be present on some of the fucose residues. 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 (Chevolot 2001).

1.2.4 Alginates

Alginic acid (Fig. 2) 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, e.g., 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 water 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 (Sartori et al. 1997, Rinaudo 2002, Pereira 2004).

1.2.5 Agar

Agar (Fig. 2) was the first colloid to be developed and 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*, *Acanthopeltis* 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, 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 (Lahaye 2001b, Bixler and Porse 2011).

1.2.6 Carrageenan

Carrageenan and agar (Fig. 2) are the principal sulphated polysaccharides produced by red seaweeds (Rhodophyta); 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 pyranosidic ring. There are about fifteen idealized carrageenan structures traditionally identified by Greek letters (Chopin et al. 1999).

The commercial carrageenans are normally divided into three main types: kappa, iota and lambda-carrageenan. 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-anhydro-galactose bridge (Bixler and Porse 2011).

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* (know in the trade as "cottonii"). *Eucheuma denticulatum* (commonly referred to as "spinosum" in the trade) is the main species for the production of iota-

carrageenan (Pereira 2013). Lambda-carrageenan is obtained from different species from the genera *Gigartina* and *Chondrus* (trade name "Irish moss") (van de Velde and de Ruiter 2002, Pereira 2013).

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 anhydro-galactose bridge of the 4-linked galactose residue, respectively DA and DA2S, which adopts the $^{1}C_{4}$-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-anhydro bridge and, therefore, the 4-linked residue adopts the $^{4}C_{1}$-chair conformation, which disturbs the helical conformation. Thus, lambda-carrageenan acts simply as a thickening agent (van de Velde and de Ruiter 2002).

1.3 Phenolic Compounds

Phenolic compounds are commonly found in plants, reportedly having several biological activities (see Table 2), including antioxidant properties. Earlier reports have revealed that seaweed extracts, especially polyphenols, have antioxidant activity (Lim et al. 2002, Kuda et al. 2005, Martins et al. 2013). Phenolic compounds are regarded for their important dietary roles as antioxidants and chemo preventive agents (Bravo 1998).

Antioxidants can be classified into two major groups; that is, enzymatic and nonenzymatic antioxidants. Some of these antioxidants are endogenously produced, including enzymes, low-molecular-weight molecules, and enzyme cofactors. Many nonenzymatic antioxidants are obtained from dietary sources. Dietary antioxidants can be classified into various classes, of which polyphenols is the largest one (Bunaciu et al. 2012).

2 Analysis of Seaweeds by Vibrational Spectroscopy

The combined use of FTIR-ATR (see Table 3 and 4) and FT-Raman (see Table 5) spectroscopy analysis can be used for the identification of main seaweed polysaccharides, namely, alginates, fucoidan, laminaran, agars, kappa-, iota-, and lambda-carrageenans (Pereira et al. 2009, 2013). Infrared spectroscopic techniques can also be used for the identification of phenolic content and total antioxidant capacity of some compounds from seaweeds (Bunaciu et al. 2012, Vijayabaskar and Shiyamala 2012, Rajauria et al. 2013).

Therefore, vibrational spectroscopy (FTIR-ATR and FT-Raman) is proposed as a useful tool for the cosmetic, pharmaceutical and food industry

Table 3. Peaks found in seaweed FTIR spectra with their attributed bonds (Adapted from Chopin et al. 1999, Pereira et al. 2009, Souza et al. 2012).

Wave numbers (cm^{-1})	**Bonds/Assignments**
3500	O-H
2960	CH_2
2900–2920	C-H (good reference for total sugar content)
2845	O-CH_3 (shoulder on the band at 2920 in highly methylated agars)
1725	COOH
1690–1695	Amide I from proteins
1640–1650	H_2O and proteins CO-NH/amide II from proteins
1605	Carboxylate anion of pyruvate
1450	Ester-sulfate
1420	Amide III from proteins
1370–1320	Ester-sulfate
1210 <1240 <1260	S=O of ester-sulfate (good indicator for total sulfate content)
1180	P-O-C (alkyl substituent's suggesting organic phosphates)
1150	Ester-sulfate
1040–1080	Skeleton of galactans
1070	C-O of 3,6-anhydrogalactose (shoulder)
1065	Gelling type carrageenans
1040	C-O of ester-sulfate and hydroxyl
1037–1071	Symmetric C-O vibration associated with a C-O-SO_3 of heterofucans
1020	Non-gelling type carrageenans
1000–1200	Sulfates and floridean starch
970–975	Galactose: peak with alkali modified iota carrageenan, small peak with unmodified iota, and also present in agars
930–940	Vibrations of the C-O-C of 3,6-anhydrogalactose
905	C-O-SO_4 on C_2 of 3,6-anhydrogalactose (shoulder)
890–900	Unsulfated β-D-galactose (or with 6–0-methylgalactose or with pyruvate); agar specific band
867	C-O-SO_4 on C_6 of galactose (shoulder, indicates precursors)
845–850	C-O-SO_4 on C_4 of galactose/floridean starch
825–830	C-O-SO_4 on C_2 of galactose (narrow when xi-carrageenan present)
820	Galactose 6-sulfate
815–820	C-O-SO_4 on C_6 of galactose
805	C-O-SO_4 on C_2 of 3,6-anhydrogalactose
790	Characteristic of agar-type in second derivative spectra
730–750	C-S/C-O-C bending mode in glycosidic linkages of agars
717	Characteristic of agar-type in second derivative spectra/C-O-C bending mode in glycosidic linkages of agars
705	C-O-SO_4 on C_4 of galactose
580	S-O in sulfated galactans

Table 4. Identification of Carrageenan types by Infrared Spectroscopy (Adapted from Chopin et al. 1999, Pereira et al. 2009).

Wave numbers (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 C_2 of 3,6-anhydrogalactose	DA2S	–	–	+	–	–	+	–	–
890–900	Unsulphated β-D-galactose	G/D	–	–	–	–	+	–	–	–
867	C-O-SO3 on C_6 of galactose	G/D6S	–	+	–	+	–	–	+	–
845	C-O-SO3 on C_4 of galactose	G4S	+	+	+	+	–	–	–	–
825–830	C-O-SO3 on C_2 of galactose	G/D2S	–	–	–	+	–	+	+	n
815–820	C-O-SO3 on C_6 of galactose	G/D6S	–	+	–	+	–	–	+	–
805	C-O-SO3 on C_2 of 3,6-anhydrogalactose	DA2S	–	–	+	–	–	+	–	–

–, absent; +, medium; ++, strong; +++, very strong; s, shoulder peak; n, narrow peak.

Table 5. Identification of Carrageenan Types by Raman Spectroscopy (adapted from Pereira 2006, Pereira et al. 2009).

Wave numbers (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	Un-sulphated 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.

to check the phycocolloid quality of a raw seaweed material by a quick and non-destructive method (Pereira 2004, Pereira et al. 2009).

2.1 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 (1971) 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, Chandia and co-workers, in work with specimens of *Lessonia* genus, assign both bands to G units (Chandia et al. 2001, 2004). Filipov and Kohn (1974) 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 Sakugawa and collaborators (2004) 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 mannurate concentration of calcium alginate and the 1025 cm^{-1} is attributed to the OH bending of guluronate (Sakugawa et al. 2004).

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* (Pereira et al. 2013). However, the FTIR spectra of *Saccorhiza polyschides* show an intense broad band centered at 1025 cm^{-1}, indicating that the samples considered are particularly rich in guluronic acid. According to several works (Skriptsova et al. 2004, Torres et al. 2007, Sahayaraj et al. 2012, Pereira et al. 2013), the spectrum of *U. pinnatiffida* (old adult thallus) indicates that the relative amounts of both mannuronate and guluronate residues are similar.

The spectra presented by Pereira and co-workers (2013), suggesting higher values of guluronic than mannuronic acid blocks in *Padina pavonica* and similar amounts of both mannuronate and guluronate residues in *Sargassum vulgare*, are in accordance with other published works (Skriptsova et al. 2004, Torres et al. 2007, Sahayaraj et al. 2012).

Some brown algae, such *Saccorhiza polyschides* and *Undaria pinnatifida*, also exhibit 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* also exhibit a broad band

in this region (around 1195–1237 cm^{-1} for *Padina* and 1210–1280 cm^{-1} for *Sargassum*) assigned to (S=O). However, *Sargassum vulgare* contains a larger amount of fucoidan than *Padina pavonica* (Pereira et al. 2013). According to Camara and co-workers (2011), characteristic sulfate absorptions were identified in the FTIR spectra of heterofucans: bands around 1239–1247 cm^{-1} for asymmetric S=O stretching vibration and bands around 1037–1071 cm^{-1} for symmetric C-O vibration associated with a C-O-SO_3 group. The peaks at 820–850 cm^{-1} were assigned to the bending vibration of C-O-S. However, *Sargassum vulgare* and *Padina pavonica* contain little amounts of laminaran (Peat et al. 1958, Nelson and Lewis 1974, Rioux et al. 2010, Jiao et al. 2011).

2.2 Identification of Ulvan

A typical infrared spectrum of ulvan shows strong absorbance at about 1650, 1250, and 1070 cm^{-1} and small ones at about 1400, 850, and 790 cm^{-1} (Ray and Lahaye 1995, Robic et al. 2009). Some of these bands are easily assigned to carboxylate groups and to sulfate esters. Carboxylate groups show two bands, an asymmetrical stretching band near 1650 cm^{-1} and a weaker symmetric stretching band near 1400 cm^{-1}, and sulfate esters show a major band at about 1250 cm^{-1}. Two other bands at 850 and 790 cm^{-1}, likely related to sugar cycles, are also observed. The 1200–1000 cm^{-1} region is dominated by sugar ring vibrations overlapping with stretching vibrations of (C–OH) side groups and the (C–O–C) glycosidic bonds vibration. All ulvan spectra presented a maximum absorption band at around 1055 cm^{-1} which are likely due to C–O stretching from the two main sugars, rhamnose and glucuronic acid: their individual IR spectra present a maximum at the same wavenumber, 1055 cm^{-1} (Robic et al. 2009).

2.3 Identification of Agar and Agarocolloids

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, between 1210 to 1260 cm^{-1} (Chopin et al. 1999), is much stronger in carrageenan than in agar. Especially in the anomeric region (700–950 cm^{-1}), agar and carrageenan show several similar bands (see Table 2). Thus, the strong IR band at 930 cm^{-1} assigned to the presence of 3,6-anhydro-galactose was common to agar and carrageenans; the band at 890 cm^{-1} corresponded to anomeric CH of β-galactopyranosyl residues and Raman bands at 770 and 740 cm^{-1} (strong in the FT-Raman spectra and weak in the FTIR-ATR) are assigned to the skeleton bending of pyranose ring (Matsuhiro 1996, Pereira et al. 2003), both in agar and carrageenans. Also, the bands at 1010–1030 cm^{-1}

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* (Rhodophyta) is agar (Pereira et al. 2003, 2013).

A typical FT-Raman spectrum of agar shows a strong band centered at 837 cm^{-1}, which is absent in the FTIR spectra (Pereira et al. 2003, 2013). According to Matsuhiro (1996), 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 (Rhodophyta) presents a complex agar-like sulfated galactan. These polysaccharides belong to the agar group, being agarose derivatives with a rather high content in sulfate groups and with a reduced amount of 3,6-anhydro-L-galactose residues (700–950 cm^{-1}) (Usov and Elashvili 1991, Pereira et al. 2013).

2.4 Identification of Carrageenan

In the works of Pereira and co-workers, the FTIR-ATR and FT-Raman spectra of *Kappaphycus alvarezii* were compared with those of commercial kappa-carrageenan (Pereira et al. 2003, 2013). The spectra of the ground seaweed show the same 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) (Pereira 2004, Pereira et al. 2009).

According to Pereira et al. (2003, 2013) the spectra of iota-carrageenan and of *Calliblepharis jubata* show 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 around 805 cm^{-1} in both IR and Raman spectra. This band, indicating the presence of sulphate ester in the 2-position of the anhydro-D-galactose residues (DA2S), is a characteristic band of the iota-carrageenan (Pereira 2004, Pereira et al. 2009).

The vibrational spectra of lambda-carrageenan and ground *Chondrus crispus* tetrasporophytes indicate high sulphate content, evidenced from the presence of a broad band between 820 and 830 cm^{-1} in FTIR-ATR spectra (see Table 4 and 5). The *C. crispus* and lambda-carrageenan FT-Raman spectra show the two combined weak bands between 815 and 830 cm^{-1} (Pereira 2004, Pereira et al. 2009).

2.5 Identification of Phenolic Compounds

The great antioxidant capacity of the phenolic compounds, from certain seaweeds (e.g., *Turbinaria conoides, T. ornata, Himanthalia elongata*—Phaeophyceae; and *Gracilaria follifera*—Rhodophyta), when compared with the standard Gallic acid activity, was evidenced in some recent works (Meenakshi et al. 2009, Devi et al. 2011, Bunaciu et al. 2012, Vijayabaskar and Shiyamala 2012, Rajauria et al. 2013).

The FTIR spectrum of the standard Gallic acid contain ten major peaks, at approximately 3366, 3283, 3065, 2654, 1703, 1618, 1541, 1449, 1099 and 1026 cm^{-1}. The absorption peaks observed for hydroxyl groups (around 3300–3500 cm^{-1}) and aromatic ring (around 1450–1470 cm^{-1} and 2850–2960 cm^{-1}) in the spectra of *T. ornata* also suggest the presence of phenolic compounds (Vijayabaskar and Shiyamala 2012).

In FTIR analysis of seaweed (*T. conoides*) extracts, the characteristic absorption of sulphate and carboxyl groups were identified. The peaks at 3537–3396 and 3419 cm^{-1} and 3419 cm^{-1} were assigned to the stretching vibration of O-H of strong relative strength in axial position, the stretching vibration of C-H, and C=O stretching mode, respectively. Bands at 3536–3440 cm^{-1} correspond to stretching vibration of O-H (Devi et al. 2011).

3 Conclusion

In respect to the methodology used in phycocolloid analysis, the development of Fourier transform infrared spectroscopy (FTIR) and of Fourier transform laser Raman spectroscopy (FT-Raman) has produced great advances in structural study of polysaccharides (Chopin and Whalen 1993, Cáceres et al. 1996, Matsuhiro 1996, Pereira 2006, Pereira et al. 2009).

FT-Raman spectroscopy in the solid state gives well-defined characteristic spectra of phycocolloids (Matsuhiro 1996, Pereira et al. 2003, Pereira 2006, Pereira et al. 2009). The analysis of ground-dried seaweed by FT-Raman possesses all the great advantages of the analysis of ground algal material with FTIR-ATR, and is a rapid and simple technique; it requires only small amounts of non-manipulated samples, which allows the most accurate determination of the native composition of the algal polysaccharides and phenolic compounds (Pereira et al. 2009, Bunaciu et al. 2012, Pereira et al. 2013).

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